1936

The influence of the phosphate-calcium ratio and of humates of iron on chlorosis in Lemna

Dale Harold Sieling
Iowa State College

NOTE TO USERS

THE INFLUENCE OF THE PHOSPHATE-CALCIUM RATIO AND OF HUMATES OF IRON ON CHLOROSIS IN LEMNA.

By

Dale Harold Sieling

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Soil Chemistry

Approved:

Signature was redacted for privacy.

In charge of Major work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

1936

TABLE OF CONTENTS

TABLE OF CONTENTS
(continued)

ACKNOWLEDGMENTS

The author wishes to acknowledge the professional and courteous assistance of Dr. Norman A. Clark in the preparation of this thesis. Dr. Clark's basic outline for this work, his helpful criticism, and his suggestions are appreciated.

INTRODUCTION

Organic matter is the characteristic material in soils. The practical agriculturalist has observed for centuries the presence of large amounts of organic matter in productive soils. The "humus" content of soils has long been considered a fairly accurate indicator of their fertility. Worn-out or naturally unproductive soils have been made productive by applications of organic composts such as manures or peat, and it is well known that one of the cardinal purposes of all crop-rotations is to increase the organic content of the soil. The investigation of the humus therefore becomes one of the most important considerations to the research worker in crops and soils.

In recent years investigators have recognized that the influences of organic matter on the soil and on plant growth are numerous and of great variety. These effects may be conveniently separated into (a) the direct influence of organic matter on the soil, and (b) the influence of organic matter in plant nutrition. From the standpoint of the soil itself, as a natural historical body, organic matter serves as a factor in differentiating a soil from simple geological deposits either partly or wholly disintegrated; it facilitates drainage and aeration; it promotes microbio-

logical activity, increases the water holding power, and aids in preventing the widely extended erosion which has become one of the principle economic problems of to-day.

From the standpoint of the plant, the question whether organic matter acts directly as an essential substance for plant growth or merely functions as a stimulant, either directly or indirectly, has been for some time a subject for experimental investigation. For a number of years it was believed that organic composts, such as partially decomposed manure or peat, contained organic substances that were essential for plant growth, in the same manner that vitamins are essential to normal animal development. To these unidentified substances Bottomley (18, 19) assigned the name "auximones"; but, after many investigators had succeeded in growing a large variety of plants in nutrient solutions devoid of organic matter, the term "auximone" lost its original significance.

The soil, as a medium for plant growth, presents a very complex system of variables. The number of these variables may be materially reduced by growing plants in nutrient solutions under controlled environmental conditions. Although relatively simple, nutrient solutions may serve as a basis for establishing some of the underlying principles of plant nutrition, and they have been used extensively for this purpose.

Nutrient solutions which contain the proportions of essential inorganic elements for optimum plant growth are said to be physiologically balanced. This physiological balance of the solution has been shown to depend upon the species of plant and upon environmental conditions.

The acid or alkaline reaction of the solution is also an important factor to be considered when any investigation of physiological balance is being made. Investigators observed that many plants which grew normally in a given solution at a pH between 4.0-5.0 became dwarfed and badly chlorotic when the pH was increased to a value of 6.0 or higher. This chlorotic condition has been attributed to the unavailability of inorganic iron in neutral or alkaline reactions; it could usually be prevented if organic iron compounds, such as ferric citrate or ferric tartrate, were used instead of inorganic iron compounds. The addition of "humus" extracts to solutions containing inorganic iron was also found to be effective in preventing chlorosis in alkaline reactions. To explain this humus action the theory was advanced that complex organic iron compounds were formed between some constituents of the humus and the inorganic iron salts, and that these organic complexes were able to furnish iron in an available form for plant utilization in neutral or alkaline reaction.

Other investigators observed that inorganic iron

compounds were able to support normal development of plants in nutrient solutions of neutral and alkaline reactions, when ammonium-nitrogen was substituted for the more commonly used nitrate-nitrogen.

Olsen (98) attributed the appearance of chlorosis in plants grown in nutrient solutions at pH 6.0-7.0 to the high phosphate-calcium ratios of the solutions employed. He used a modified Knop's solution and reported that, if the phosphate-calcium ratio was reduced to one fifth the usual amount by lowering the phosphate added, the resulting solution supported normal growth of maize plants at pH 6.0-7.0.

The purpose of the investigation reported here was to study systematically the influence of soil organic matter on the growth of Lemna major at different reactions, by using various quantities of alkaline humus extracts in sterile nutrient solutions, and to include the effect of humus substances upon the availability of iron for plant growth at these reactions. The optimum phosphate-calcium ratio in nutrient solutions was investigated for the growth of Lemna major in a wide range of hydrogen ion concentration, and Olsen's correlation of this ratio with chlorosis was examined for plants grown under sterile conditions.

HISTORICAL

The study of the factors involved in plant nutrition and plant growth has inspired a number of investigators in interesting and progressive research. Although only a few phases of plant nutrition and plant growth have been investigated here, associated phases will be briefly reviewed.

Factors

Stiles (122) and Stiles and Jörgenson (123) stated that for interpreting data concerning plant growth consideration must be given to:— (a) solution technique, (b) germination of the seed, (c) heredity of the plant, (d) the nutrient solution used, and (e) climatic factors. In addition to these Brenchley (23) stressed the importance of food supply, water supply, light, temperature, and atmospheric conditions as factors that had definite effects upon the rate and quality of plant growth. For best results in germination and growth of plants Livingston (86) emphasized the importance of the seed used, types of salts in the medium, salt proportions, total concentration, and temperature. Along with the external factors, light and temperature, Hanna (63) considered humidity and precipitation of

great importance. Due to the close relationship of the effects of light, temperature, and humidity upon plant growth, Blackman (12) introduced the word "interrelated" for these factors.

Concentration

Breazeal (22) observed that, if the solution were continuously renewed, wheat plants gave their maximum growth in the highest concentration of the nutrient solution he employed. His solution contained 15, 75, 155, 750, and 1,550 ppm. of total solutes. To further his investigation, the same plant was used and also the same concentrations, but the solutions were renewed only at three and one half day intervals. Under these conditions the maximum growth was obtained in the solution having a concentration of 155 ppm. Using barley for experimentation, Hall, Brenchley, and Underwood (61) observed that the barley plant increased in growth in proportion to the concentration of solutes; however, Stiles (121) (122) obtained very little difference in growth response in the solutions of different concentrations.

Expressing concentration in terms of osmotic pressure, Shive (116) (117) reported that both wheat and buckwheat gave the best growth in solutions having a total concentra-

tion of 1.75 atmospheres, and the growth was poorer in solutions having a total concentration of 0.1 and 4.0 atmospheres. Using two species of hybrid tobacco as test plants, Ayres (8) obtained superior growth when the total concentration of the nutrient solution was relatively low.

Pember (102) obtained optimum response for barley in solutions with high concentrations. Hoagland (68) grew barley in solutions with total concentrations of 200, 800, 2,500, and 6,000 ppm. and reported optimum growth in the solutions containing 800 and 2,500 ppm. and inferior growth at the high and low concentrations. Hoagland also grew barley in solutions in which the total concentration was 0.07, 0.58, 0.90 and 1.70 atmospheres, expressed in terms of osmotic pressure, and found that maximum growth occurred at concentrations of 0.58 and 0.90 atmospheres.

Reed and Haas (107) reported that the growth of the African sour orange increased progressively from a total concentration of 364 ppm. to 2,181 ppm., but decreased as the concentration became higher than 2,181 ppm.

Using Spirodella polyrhiza (Lemna major) as the experimental plant, Saeger (110) found that very poor growth resulted when Detmer's solution or Knop's solution was used; however, if these solutions were diluted to give concentrations of 1/5, 1/10, 1/50, and 1/150 of the original concentrations, maximum growth was obtained at the 1/10 concentra-

tion, and the growth decreased as the concentration became greater or smaller.

Eisenmenger (47) found little variation in the elongation of wheat roots grown in solutions with a rather wide range of total concentration. According to Sideris, Krauss, and Masunga (119) pineapple plants respond most favorably to nutrient solutions having a total concentration approximately equal to the concentration of the cell sap, with the growth decreasing considerably as the concentration was changed.

This entire group of investigators have observed that total concentration within certain limits does not cause variation in plant growth, and it becomes important only when the concentration exceeds these limits.

Physiological Balance

Osterhout (99, 100, 101) formed the opinion that correct salt proportion rather than total concentration was the first essential of a good nutrient solution for plants. Gile (55) suggested that variations of the lime-magnesia ratio in nutrient solutions of high concentrations were unsatisfactory for the growth of rice--the variation acted in further unbalancing a solution which was already unbalanced--but that such a variation in dilute solutions was

more effective. Physiological balance in nutrient solutions was considered more important than total concentration by Ayres (8); and this was also the conclusion of Sideris, Krauss, and Masunga (119).

The first systematic study of the physiological balance of nutrient solutions was conducted by Tottingham (129). He used the wheat plant in solutions containing four inorganic salts with a total concentration of 0.05, 2.5, and 8.15 atmospheres, expressed as osmotic pressure. Each concentration was investigated with a series of eighty four solutions containing varying molecular proportions of each salt. Using dry weight as a criterion of growth, Tottingham (129) obtained optimum growth in the solution containing 0.0130 mols of KH_2PO_4, 0.0145 mols of $MgSO_4$, 0.0144 mols of $Ca(NO_3)_2$, 0.0049 mols of KNO_3, and a few drops of colloidal ferric phosphate per liter of solution. Shive (116, 117) carried on the same sort of systematic investigation and observed that three salt nutrient solutions could be proportioned molecularly to give growth equal to, or better than that obtained by using Tottingham's (129) best four salt solution; and that wheat and buckwheat required solutions having different salt proportions for maximum growth.

The use of different salts as the sources of the essential elements was studied by Livingston and Tottingham (87); their investigation showed that mono-calcium phosphate,

potassium nitrate and magnesium sulfate could be balanced in nutrient solutions which would be as effective for growth of wheat as the usual combination of primary potassium phosphate, calcium nitrate, and magnesium sulfate. Gericke (52) grew wheat plants in dilute solutions of KNO_3 for 24 hours, the second day in $MgHPO_4$, the third day in $CaSO_4$, and this rotation was continued for four weeks; the growth obtained by this procedure was almost equal to that of a controlled solution containing all the ions, but was superior to single salt solutions having other combinations of the same ions.

The committee of Biology and Agriculture of the National Research Council recommended the use of solution III containing $Ca(H_2PO_4)_2$, KNO_3, and $MgSO_4$ for the growth of seedlings. Clark (34, 36) reported that the growth of Lemna polyrhiza was inhibited by this solution; however he found that the salt proportions could be varied to give excellent growth. A liter of Clark's best solution for Lemna polyrhiza contained 0.4 millimols of calcium as monocalcium phosphate, 8 millimols of potassium as potassium nitrate, 1 millimol of magnesium as magnesium sulfate, and 0.01 millimols of iron as ferric chloride. Using a closely related plant, Lemna minor, Wolfe (137) reported good growth in Shive's best solution for wheat. Wolfe (137) attributed Bottomley's (19, 20) failure to get successful

growth of Lemna major and Lemna minor in Detmer's and Knop's solutions to the unbalanced condition of these solutions.

The salt proportions required to produce maximum response in the development of wheat in the seedling, vegetative, and fruiting stages were shown by Sewell (114) to be different for each. Johnson (75) reported that, of the six types of nutrient solutions recommended by the National Research Council, types I and II were better suited for the growth of Irish Cobbler potatoes than any of the other types, if dry weight was taken as an indicator of growth.

That physiological balance of the nutrient solution is one of the most important factors to be considered when plants are being grown in nutrient solutions is shown by the observations of these investigators. Each plant apparently requires a solution of fairly definite composition for its optimum growth, although good growth may be obtained in other solutions. The physiological balance of the nutrient solution depends primarily upon the plant used, the nature of the salts, and the stage of growth of the plant.

Phosphate-Calcium Ratio

One of the phases of physiological balance that has

been studied in this investigation is the effect of the phosphate-calcium ratio of the solution on the growth and chlorosis of green plants at various reactions. This particular factor has been stressed by Olsen (92) for the production of normally green plants at pH 6.0-7.0 in inorganic solutions. He observed that a number of plants became cholorotic at pH 6.0-7.0 if they were grown in a modified Knop's nutrient solution which contained in one liter, 2.54 millimols of calcium as calcium nitrate, 1.10 millimols of phosphate as primary potassium phosphate, and 7.0 milligrams of iron as ferric chloride, and had a phosphate-calcium ratio of 0.432; however, normally green plants and good growth were obtained at pH 5.0 and 8.0. The normal growth at pH 5.0 had been obtained by several investigators, but growth at pH 8.0 had not been accounted for by any previous investigations. Olsen (92) analyzed the nutrient solutions which had been adjusted to pH's of 4.5, 6.0, 7.0 and 8.0 and observed that the solution at pH 8.0 contained only about one fifth of the phosphate that had been originally added, while the solutions at pH 6.0-7.0 contained approximately 95% of the original. He then lowered the phosphate-calcium ratio in the solution to 0.086, by reducing the millimols of phosphate one fifth and by keeping the concentrations of the other salts at the same level. Maize plants which were grown in this adjusted

solution for 18 days produced maximum growth at pH 7.0 with no apparent chlorosis appearing at any reaction of solution from pH 4.0 to 8.0. From these data Olsen (92) concluded that phosphate was the ultimate cause of chlorosis, and stated that it was due to the precipitation of ferric phosphate within the vascular bundles of the plant; this could be prevented by reducing the phosphate to such a level that the calcium in the solution was always in excess compared with the phosphate.

Since this particular point has not been noted before, it was thought advisable to study the data of previous investigators from this point of view. Only those investigations in which inorganic iron was used are considered as inclusion of organic matter introduces further complications.

Gile (54) concluded that both alkalinity and high calcium were responsible for chlorosis in pineapple plants, because neither condition would cause chlorosis when acting alone. When rice plants were grown by Gile and Carrero (56) in nutrient solutions with a phosphate-calcium ratio of 0.044 chlorosis soon developed; but doubling the phosphate of this solution did not increase the severity of the chlorosis. Willis and Carrero (136) observed that application of nitrates or ammonium phosphate to sandy soils was followed closely by the appearance of chlorosis

in rice plants. The data of Salter and McIlvane (111) showed that wheat, soybeans, alfalfa, and corn seedlings gave very poor growth when grown in solutions having pH's of 6.97 and 7.71 and a phosphate-calcium ratio of 7.2.

Using a nutrient solution with a phosphate-calcium ratio of 0.135 McCall and Haag (89) reported that all solutions, with reactions more alkaline that pH 4.02, caused marked chlorosis and depression of the growth of wheat seedlings. Tarr and Noble (124) used a nutrient solution, having a phosphate-calcium ratio of 3.28 and buffered at 1.0 pH intervals from pH 3.0 to 8.0 with potassium acid phthalate, for the growth of wheat, corn, and soybean seedlings; in all cases severe chlorosis occurred when the reaction of the solution was pH 6.0 or above. Bryan (25) observed that soybeans showed maximum growth at pH 6.0-7.0 in a nutrient solution having a phosphate-calcium ratio of 0.635. Bryan (25, 26, 27) also used a modified Crone's solution for growth of several test plants. All of the calcium in this modified Crone's solution was obtained from the relatively insoluble $Ca_3(PO_4)_2$ and $Ca\ SO_4.2H_2O$ by shaking quantities of these salts with distilled water and then filtering off the undissolved residue; to the resulting solution was added 0.25 grams of secondary sodium phosphate per liter and this would undoubtedly cause the phosphate-calcium ratio to be

rather high. This nutrient solution was employed as the growth medium for soybeans, oats, wheat, alfalfa, alsike clover, and red clover for a period of two months, during which time the solutions were renewed daily. In all cases the maximum growth, on the basis of dry weight, was obtained at reactions of pH 6.0 to 8.0.

Brown (24) grew wheat plants in sand cultures containing various proportions of calcium and phosphate. At pH 7.0 in a solution having a phosphate-calcium ratio of 0.247 the plants were normally green and produced good growth; however in another solution at pH 6.0 having a phosphate-calcium ratio of 0.663 the plants became chlorotic after the seedling stage. With a phosphate-calcium ratio of 2.0 in the nutrient solution used, Tottingham and Rankin (131) observed that chlorosis appeared in wheat plants grown at pH 6.4 but that normal chlorophyll development occurred at pH 7.5. By increasing the calcium in solution until the phosphate-calcium ratio was 0.070, Chapman (30) found that less chlorosis developed in Brassica alba at pH 6.0 to 7.4 than at the same reactions when the ratio was 0.65.

Arndt (5) reported that corn became chlorotic in solutions with a phosphate-calcium ratio of 0.66, but developed into normally green plants when the ratio was reduced to 0.064. Although all plants were chlorotic, Sideris and Kraus (117) obtained good growth of maize

plants in solutions high in iron regardless of the phosphate content; however, in solutions containing low concentrations of iron, the maximum growth was obtained in the low concentrations of phosphate. Spencer and Shive (120) observed that the appearance of chlorosis in Rhododendron was correlated with the increased proportions of calcium nitrate in the solution.

Adams (1) concluded that calcium was the "key" element in the nutrition of soybeans; the intake of P_2O_5 and K_2O was regulated by the calcium sulfate of the superphosphate added as fertilizer.

The influence of the phosphate-calcium ratio upon the growth of several plants is summarized in Table I.

Ammonium Ion

Another method for prevention of chlorosis of green plants grown at neutral or alkaline reaction in inorganic media has been employed by several investigators. Jones and Shive (80) observed that by substituting ammonium sulfate for the more commonly used potassium nitrate, good growth with no chlorosis was obtained for soybeans at pH 6.0. Tiedjens and Robbins (127) grew tomatoes at pH values from 3.0 to 8.0 in Jones and Shive's (80) solution containing ammonium sulfate as the source of nitrogen. Although the

Table I. Phosphate-calcium ratio and chlorosis of plants.

Name of Investigator	Plant Studied	Phosphate-Calcium Ratio	pH	Observation
Gile and Carrero (56)	rice	0.044	alkaline	became chlorotic
Arndt (5)	corn	0.064	neutral	remained green
Chapman (30)	Brassica alba	0.065	6.0-7.4	slight chlorosis
Olsen (92)	maize	0.086	4.5-8.0	no chlorosis, optimum growth at pH 7.0
McCall and Haag (89)	wheat	0.135	4.02-7.0	chlorotic and poor growth
Spencer and Shive (120)	Rhododendron	0.206	not given	very chlorotic
Brown (24)	wheat	0.247	7.0	normally green
Olsen (92)	maize	0.432	4.5-8.0	chlorotic at pH 6.0-7.0, normally green at 4.5 and 8.0
Bryan (25)	soybean	0.635	3.0-10.0	optimum growth at pH 7.0
Chapman (30)	Brassica alba	0.65	6.0-7.4	chlorotic
Arndt (5)	corn	0.66	neutral	chlorotic
Brown (24)	wheat	0.663	6.0	chlorotic
Spencer and Shive (120)	Rhododendron	0.90	not given	normally green
Tottingham and Rankin (131)	wheat	2.00	6.4-7.5	chlorotic at pH 6.4 normally green at 7.5
Tarr and Noble (124)	wheat, corn, soybean	3.28	3.0-8.0	chlorotic above pH 6.0
Salter and McIlvane (111)	wheat, corn soybean alfalfa	7.2	3.0-7.2	very poor growth above pH 6.0

phosphate calcium ratio of this solution was 1.48, no chlorosis was evident at any reaction used; maximum growth was obtained at pH 7.0-8.0 with growth decreasing as the solutions became more acid. A duplicate series containing potassium nitrate as the source of nitrogen was investigated along with this ammonium sulfate series. The plants grown in all solutions of the nitrate series at pH 6.0 or higher gave distinctly chlorotic plants and poor growth. Tiedjens and Robbins (127) obtained the same results in these two solutions with peppers, cucumbers, onions, lettuce, and beets, and when the phosphate-calcium ratio of the ammonium-nitrogen series was increased to 4.3 good growth was obtained for tomatoes, apple and peach trees, soybeans, and cucumbers at pH 7.0. Tiedjens and Blake (126) reported that luxuriant growth of peach trees was obtained at pH 6.5 to 8.5 if ammonium salts were used as the source of nitrogen and that severe chlorosis resulted at pH's above 6.5 when nitrate nitrogen was used. Davidson and Shive (43) confirmed these findings of Tiedjens and Blake (126) concerning the peach tree.

Auximones

The influence of organic matter in plant nutrition has been investigated extensively in the past few years

and various results and conclusions have been reported. Livingston (85) observed that the growth of wheat was stimulated quite substantially by the addition of manure to the inorganic nutrient solutions. Using wheat as a test plant in 66 different solutions, Schreiner and Skinner (113) reported that additions of 50 ppm. of pure organic composts to nutrient solutions gave variable results; in most cases stimulation of growth resulted, especially if the organic compound contained nitrogen, and this stimulation was more pronounced when nitrates were absent.

Bottomley (12, 21) and his co-worker Mockeridge (93, 94) published a series of papers from 1912 to 1924 concerning the effect of organic matter on the growth of plants in nutrient solutions. Bottomley treated peat with aerobic bacteria and extracted this compost with distilled water to obtain a black humus substance which he called "bacterized" peat extract. He observed a marked stimulation of growth for several plants when this peat extract was added to the soils in which the plants were growing. In nutrient solutions to which this extract had been added, wheat and other seedlings showed some stimulation, but not as much as expected. This smaller response was traced to the presence of the stimulating substance in the seed. Wheat seedlings from which the seed was removed shortly after germination, were grown in two series of Detmer's nutrient

solutions, one of which contained no organic matter and the other bacterized peat extract. In all cases the seedlings in the solution containing the peat extract grew very well, while those in the inorganic media soon died; thus Bottomley concluded that the seed contained some essential substances without which the seedlings would not grow in inorganic media, and that these compounds were present in the peat extract. To these unidentified essential substances he gave the name "auximones", which means growth promoting. Bottomley, (19, 20, 21) then grew Lemna major and Lemna minor in both Detmer's and Knop's solutions made up of inorganic salts only, and in duplicate solutions to which had been added 368 ppm. of organic matter as "bacterized" peat extract. The difference in growth and reproduction was marked; the plants which grew in the solutions containing organic matter gave as much as 20 times the reproduction and 60 times the dry weight as those in strictly inorganic solutions. Mockeridge (93, 94) attributed in part the growth promoting power of bacterized peat to its nucleic acid content, and gave as evidence the fact that rotted manure was more effective in promoting growth and also contained more nucleic acid than did fresh manure.

These conclusions of Bottomley were not accepted as final by a number of investigators. Clark (34, 36) and

Clark and Roller (39, 40) showed that "auximones" were not essential to plant growth by growing Lemna major successfully for several years at pH 4.7-4.9 in nutrient solutions containing no organic matter. These investigators found in some cases that addition of small quantities of sterile organic matter to sterile cultures of Lemna major depressed rather than stimulated the reproduction, while the addition of non-sterile organic matter usually caused a marked stimulation of growth. They also observed that additions of sterile urea to sterile cultures of Lemna major caused a depression of growth while the effects of acetamide and creatinine were not noticeable. Saeger (110) confirmed the work of Clark and Roller (39, 40) by growing Spirodella polyrhiza (Lemna) in Knop's solution which had been diluted to one tenth the usual concentration, and found no visible depression on the growth or reproduction; when either autolyzed yeast or alkaline peat extracts were added to the solutions, the growth rate was increased greatly. Wolfe (137) observed that several pure organic substances depressed the growth of Lemna minor and that this plant could be grown successfully in purified inorganic media. Using Clark's (35) nutrient solution, Ashby (7) grew Lemna minor for several generations without any noticeable depression in the rate of reproduction or the size of the plants. This investigator also reported that 2 ppm. of organic matter

added to Clark's solution at pH 4.8 was effective in increasing frond area, cell size, and the number of chloroplasts of Lemna minor, but that additions of organic matter greater than 2 ppm. produced no increased stimulative effect.

In general, it may be stated that organic matter, although not essential to plant growth, has a stimulative effect in some cases and a depressive effect in others, depending upon the conditions of the experiment and the type of organic matter used. In sterile cultures the same substance which stimulates growth in non-sterile solutions, may not affect the plant or may even depress the growth.

Iron and Organic Matter

Olsen (97, 98) observed that addition of alkaline humus extracts to nutrient solutions of neutral or alkaline reaction had the same influence in the prevention of chlorosis and stimulation of growth of Lemna major that was noted when ferric citrate was added. This investigator concluded that the stimulative effect of organic matter in nutrient solutions is primarily due to the property of organic matter to form complex organic compounds which increase the availability of iron for plants in alkaline or neutral reaction. Olsen suggested that the iron passed directly into the plant in the form of the complex molecule—the ferric citrate. Similarly the observations of Gile (54) and Willis

and Carrero (136) indicated that calcareous soils would cause chlorosis when low in organic matter, but would produce normally green plants if their organic content was high.

Burk, Lineweaver, and Horner (28) reported that the growth stimulation of azotobacter and of tomatoes was preproportional to the iron content of the humic acids which had been added to the nutrient solutions; they were able to get greater stimulation of growth from humic acid by increasing its iron content. Organic compounds such as ferric citrate and ferric tartrate, produced the same stimulation in the growth of azotobacter. Gile and Carrero (57) observed that the organic compounds ferric humate, ferric molasses, and dried blood were not effective in prevention of chlorosis of plants grown in calcarious soils but were effective when added to nutrient solutions. These investigators concluded that organic substances were able to prevent chlorosis in water cultures but not in soil cultures because of the higher sterility of the water cultures which prevented the rapid destruction of the complex organic iron compounds by bacterial action. Hopkins (70) and Hopkins and Wann (71, 72) concluded that the addition of sodium citrate to nutrient solutions for Chlorella caused a growth stimulation by making the iron more available for assimilation. They found a different

citrate-iron ratio for each concentration of iron that gave maximum stimulation of growth. These investigators suggested that the iron-ion was the controlling factor in growth, and that addition of sodium citrate, above the optimum citrate-iron ratio, caused a depression of the quantity of iron-ion by reducing the ionization of the iron citrate molecule.

Gris (59) observed that iron was essential for the formation of chlorophyll and for the normal development of plants. Wolff (138) suggested that iron acted as a catalyst in the formation of chlorophyll in the plant. The form and amount of iron required for normal plant growth has been studied extensively but with considerable variation in the results.

Organic iron compounds such as ferric citrate, ferric tartrate, ferric oxalate, and ferric glycero-phosphate have been used in nutrient solutions for the source of iron by a number of investigators. Gile and Carrero (57) observed that rice grew well in neutral or alkaline solutions containing ferric tartrate and ferric citrate. That ferric tartrate was a suitable source of iron for orange trees in solutions at pH 5.2 to 7.5 was shown by Reed and Haas (107). Duggar (46) used a mixture consisting of ferric citrate and tri-sodium phosphate as the source of iron and obtained good growth of wheat, corn, and field peas between pH 4.5 and

pH 7.1. Deuber (45) observed that normal growth was obtained for Lemna major in Knop's nutrient solution at pH 6.5 when 2-3 ppm. of ferric citrate were added. Clark (36) reported that in solutions containing 0.6 mgm. of iron per liter as ferric citrate Lemna major became chlorotic at reactions more alkaline than pH 6.0. Lemna major and several other species of plants, according to Olsen (97, 98), developed normally in nutrient solutions at pH's 4.0 to 8.0 when 5.0 to 7.0 ppm. of iron were supplied as ferric citrate. The maximum growth of Lemna major in Clark's nutrient solution at various reactions was shown by Fly (49) to depend upon the concentration of ferric citrate. Fly grew Lemna major in solutions containing from 0.5 mgm. to 32. mgm. of iron per liter as ferric citrate and observed that the optimum reaction for growth changed progressively from acid reaction to alkaline, as the concentration of the ferric citrate was increased.

Organic matter evidently is closely related to the iron availability in plant nutrition. It can aid plant growth by acting as a carrier for the iron at neutral and alkaline reactions in which inorganic iron is relatively unavailable for plant assimilation. Whether the iron is carried directly into the plant as a complex molecule or as an ion which dissociates from a complex molecule, is not yet clear.

Inorganic Iron Salts

The use of various inorganic iron salts in nutrient solutions has received considerable comment in the past few years. Iron was usually added to the nutrient solution in the form of a definite quantity of inorganic iron salt at the time of preparation of the nutrient solutions; this, however, has been shown to be undesirable by Marsh and Shive (91). They observed that definite applications of iron at fixed intervals during any physiological stage in the growth of plants were not practicable, since the ever changing plant environment had a marked effect on the iron requirement. They suggested that the most successful method for the prevention of chlorosis in nutrient solutions was to supply iron compounds day by day as the appearance of the plant indicated.

Gile and Carrero (56) observed that ferric chloride was much more effective in preventing chlorosis of rice than was colloidal iron. This observation was confirmed by Chapman (30) who found that colloidal iron was not effective in the prevention of chlorosis of Brassica alba. Jones (76), on the other hand, found that colloidal ferric phosphate in the concentration of 0.814 mgm. per liter was able to prevent chlorosis of Marquis wheat in solution cultures.

Corson and Bakke (41) observed that wheat showed a

preference for ferric phosphate while Canadian field peas responded equally well whether ferric phosphate or ferrous phosphate was used for the source of iron. Ferrous sulfate was shown by Hartwell and Pember (64) to serve as a source of iron for rye or barley seedlings. That peas and barley required like quantities of iron was the observation of Toole and Tottingham (128). Lemna major and soybeans were found by Deuber (45) to have approximately the same iron requirement when grown in the same types of cultural solutions.

That the importance of the choice of iron salt depended upon the kind of nutrient solution employed was shown by Jones (76). Jones observed that ferric phosphate was able to prevent chlorosis in nutrient solutions containing ammonium salts as the source of nitrogen, but was unable to prevent chlorosis in solutions containing nitrates; ferrous sulfate, on the contrary, was toxic in the ammonium series but very effective in supporting normal growth in the nitrate series. These results of Jones (76) were confirmed by Jones and Shive (77, 78, 79, 80), and Barnette and Shive (10).

The effectiveness of various iron salts has been studied with special emphases on the total amount of salt required for normal growth of plants. Gile (54) observed that ferrous sulfate had a decided advantage over the same

concentration of ferric chloride for the nutrition of rice. This observation was also made by Arndt (5) who found that the amount of ferrous sulfate required to produce normal growth of corn was much smaller than the amount of ferric nitrate required. In the experiment of Tottingham and Rankin (132) wheat showed a more favorable response to ferric phosphate than to either ferric sulfate or ferrous sulfate with the same concentration of iron per liter. Corson and Bakke (41) found that wheat grew better in solutions containing ferric phosphate than in solutions containing ferrous phosphate, while Canadian field peas showed no advantage in either solution. Deuber (45) observed that Lemna major gave approximately the same growth response at pH 6.2 when iron as ferric citrate, ferric chloride, ferrous sulfate, or potassium ferrocyanide, was supplied in the concentration of 2-3 ppm. but that both Lemna major and soybeans became chlorotic when ferri-ferro cyanide was used as the source of iron. At pH 5.0 ferri-ferro cyanide was a better source of iron than an equal concentration of ferric citrate. Clark (35) also used Lemna major and found that ferric chloride in the concentration of 0.60 mgm. of iron per liter was slightly superior to ferric phosphate and ferric nitrate in the same concentrations. At the same time Clark observed that by increasing the concentration of iron above 0.6 mgm. per liter a depression in the growth of the Lemna occurred. That nutrient solutions containing 7,

14, 28, and 35 ppm. of iron produced growth of maize proportional to the concentration of the iron was shown by Sideris and Krauss (118). Barnette (9) and Sideris, Krauss, and Masunga (119) observed a marked relationship between the reaction of the solution and the availability of iron.

From these somewhat confusing reports it is clear that the iron salt most desirable for plant growth depends not only upon the kind of plant but also upon the type of nutrient solution and the total concentration of the iron. It has been observed that both the quantity of iron available and the form in which it is presented to the plant can have a marked influence upon the growth and development.

Reaction of Solution

The reaction of the nutrient solution as a factor in the growth of plants has received extensive study, but with widely divergent results and opinions. Previous to Hoagland's (66) original work concerning the effect of the concentration of hydrogen and hydroxyl ions on the growth of barley seedlings, titratable acidity and alkalinity were used as the measure of the reaction of a nutrient medium for plants. It is now accepted that titratable acidity or alkalinity is not the true measure of effective acidity or alkalinity; this is measured only by pH. Therefore, all work previous to that of Hoagland's is omitted.

The influence of the pH--or reaction--of the culture on plant growth has been found to be dependent upon many other factors. The relation of organic matter and phosphate-calcium ratio to the reaction of the cultural solution has already been discussed. Several further factors in plant growth which are influenced by the reaction of the medium need to be reviewed.

That the reaction of growth media is influenced by the growing plant has been shown by several investigators. Jones and Shive (77) and Jones (76) observed that the reaction of nutrient solutions having ammonium salts as their source of nitrogen became more acid when in contact with the plant roots, while those containing nitrates as the source of nitrogen became more alkaline if the reaction was originally acid, and became more acid if the reaction was originally alkaline. A few investigators, Hoagland (67, 69), Hixon (65), Meir and Hallstead (92), Theron (125), and Clark and Shive (32, 33), observed a decided change in the reaction of the nutrient solutions when in contact with the plant roots. This change was usually toward the most favorable reaction for growth of the test plant, and was attributed to the unequal absorption of ions.

There is undoubtedly a definite pH range of culture for every species of plant within which this species can develop normally when no other factors are limiting. Although the

literature gives very conflicting data, the limits of reaction for normal growth of the great majority of plants can be set at pH 3.0 and 9.0. Hoagland (67, 69), Duggar (46), Salter and McIlvane (111), Bryan (25, 26, 27), Tarr and Noble (124), Theron (125), Crist (42), Clark (36), Waltman (135), and Clark and Shive (31,32) have all observed that growth is seriously impaired or completely stopped for a wide variety of plants at pH's below 3.0 and above 9.0; both Hoagland (69) and Theron (125) reported that bermuda grass survives and thrives at the limiting of 9.0.

Another controversial subject is the optimum pH of the nutrient solution required for each species. Olsen (98) has shown that adjustment of the phosphate-calcium ratio to 1/5 of that of "modified" Knop's solution shifted the optimum pH for maize from between pH 4-5 to a reaction of about pH 7.0. That the optimum pH for Lemna major moves progressively from acid reaction to the alkaline reaction with increase in the proportion of ferric citrate in solution has been observed by Fly (49). Prianischnikow (106), Pirschle (103), Tiedjens and Robbins (127), Tiedjens and Blake (126), and Davidson and Shive (43) agreed that substitution of ammonium nitrogen for nitrate nitrogen in solution cultures caused the optimum reaction for growth to shift from between pH 4.0 and 6.0 to between pH 6.0 and 8.0. Other very conflicting data concerning the optimum reaction for growth of a wide variety

of plants in different kinds of solutions have been reported by Hoagland (66, 67), Salter and McIlvane (111), McCall and Haag (89), Bryan (25, 26, 27), Tarr and Noble (124), Tottingham and Rankin (131), Theron (125), Crist (42), Clark (36), Morris and Crist (95), Janssen (74), and Waltman (135).

It seems possible to conclude that the nature of the solution,, the physiological balance, the kind of salts, and the concentration are factors of importance in determining the optimum reaction for any plants. The reaction range of the culture, in which the plant can develop normally, depends largely upon the nature of the solution and the species of the plant; however, there is a marked tendency for the plant to shift the reaction of the solution to the most favorable reaction for its growth.

Temperature and Light

Blackman (12) recently designated light, temperature, and humidity as the "interrelated" factors in plant growth. This investigator stressed the importance of a balance between these external conditions as a means of securing optimum growth.

Leitch (83) defined optimum temperature as the highest temperature at which the process of growth would take place at a constant rate; for peas, this temperature was reported

to be 29° C. Rudolfs (109) observed a marked increase in the rate of germination and elongation of bean seedlings with an increase in temperature. The optimum temperature for the growth of maize seedlings was found by Lehenbauer (84) to depend upon the length of time the plant was exposed to light. He observed that if corn seedlings were exposed to high temperatures and long periods of illumination there was an initial increase in growth followed by an inactive period of growth. This observation was substantiated by Andrews and Beals (4) who found that corn grew better at a "favorable" temperature than at higher temperatures. Sideris and Krauss (118) attributed pronounced chlorosis of maize to the high temperature of the green house in which the plants were grown. Trelease and Trelease (134) compared root growth and germination of wheat in a number of different nutrient solutions at 14° C., 19° C., and 30° C., and reported that for the different temperatures certain of the solutions had nearly the same physiological value, while other solutions showed different physiological values when tested at different temperatures. This was also observed by Gericke (51) who reported that temperature had a marked influence upon the mineral requirement of wheat. McDougal (90) observed that wheat was less sensitive to high and low temperatures than was corn.1 Sunflower was found by Hanna (62) to grow at much lower temperatures than corn. The

optimum temperature for wheat was reported by Davis and Hoagland (44) to be 25° C.

The influence of light as a factor in the development of plants has been reviewed recently by Burkholder (29) and the reader is referred to his article for a more complete discussion of the subject. Burkholder (29) separated the influence of light upon plants into several individual factors such as exposure and intensity. The correlation of intensity, wave length, and time of exposure with growth is reviewed briefly here.

Garner and Allard (50) reported that although light intensity was not a factor in the attainment of the reproductive stage of growth, it was effective in controlling the vegetative growth. The growth of wheat was found by Davis and Hoagland (44) to be directly proportional to the intensity of the light when intensities of 1200 foot candles to 3000 foot candles were used. This was also the observation of Popp (105) for soybeans when dry weight was taken as a measure of the growth. Popp found that the thickness of the stem of soybeans was directly proportional to the intensity of the light employed, but that the height of the plant increased progressively as the intensity increased from 26 foot candles to 560 foot candles, and then decreased as the intensity increased. This observation was substantiated by Shirley (115) who reported that growth of sunflowers and

Galinsoga was directly proportional to the intensity of winter sunlight, but that growth was depressed when these plants were exposed to more than fifty per-cent of full summer sunlight. Ashby (6) grew Lemna minor in nutrient solutions exposed to light intensities of 1400, 700, and 300 foot candles and observed that for a fixed period of illumination maximum growth was obtained at 700 foot candles; however, he concluded that the optimum intensity was probably between 700 and 1400 foot candles. Maximum reproduction of Lemna major was observed by Clark (35) to be at the higher intensity when the plants were exposed for equal intervals of time to light intensities of 400 and 900 foot candles.

That increased light intensity had a marked physiological effect on green plants was observed by Gile (54). He concluded that strong light increased chlorosis by more rapid destruction of the chlorophyll. Ingalls and Shive (73) also noted an increase of chlorosis of plants exposed to high light intensities, but attributed this injury to the increase in the reaction of the cell sap which would not allow the proper translocation of the iron within the plant. Gericke (53) reported that high intensities of light caused marked etiolation and more mature but smaller plants than low intensities.

The importance of wave length of the light to which plants were exposed was shown by Popp (104) who observed

that sunlight with a wave length of from 290 millimicrons to 720 was the most effective source of light for plants. This investigator also observed that when plants were grown in sunlight from which the rays shorter than 472 millimicrons were excluded there was a marked etiolation of plants; the appearance of the plants was very similar to plants grown in greatly reduced sunlight. These observations of Popp (104) were confirmed by Shirley (115) and Blackman (12). Both of these investigators reported that maximum plant growth was obtained in complete sunlight and that plants grew more efficiently without red rays than without blue rays.

Eltinge (48) rayed plants with the complete spectrum of quartz ultra-violet light and observed that severe injury resulted; however, if plants were exposed to ultra violet light, from which all rays shorter than 313 millimicrons were excluded by screening, no injury to the plants was shown and in some cases the plants were stimulated in their growth.

Sayre (112) noted that the effectiveness of radiant energy in the formation of chlorophyll appears to increase with the increase of wave lengths up to 680 millimicrons and then to end abruptly. Sayre also observed that all wave lengths between 300 millimicrons and 680 were effective in formation of chlorophyll provided the energy value was

sufficient, and that for equal energy values the red rays were more effective than the green and the green more effective than the blue.

The length of exposure of the plant to light has been shown by Garner and Allard (50) to be of vital importance in the attainment of the flowering and fruiting stage of growth. These investigators observed that plants could reach the stage of reproduction only if the light period to which they were exposed fell within certain limits; on the other hand, vegetative growth was found to be directly proportional to the length of exposure of the plant to light. They suggested the term "photo-period" to designate the most favorable length of the day for each organism.

Davis and Hoagland (44) also found that growth was directly proportional to light exposure. This observation was confirmed by Trelease and Livingston (133). Ashby (6) reported that the growth rate of Lemna minor was greater for continuous illumination than for shorter periods. He also reported that growth in 2 hour alternate illumination for 24 hours did not differ greatly from that in 12 hour illumination. That the rate of growth and reproduction of Lemna major increased progressively as the length of exposure to light increased was reported by Clark (35); this investigator observed, however, that the plants grown in periods of illumination of much over 15 hours became unhealthy in

appearance, while those exposed to 15 hours lighting presented a much better natural vigor.

These reports confirm and extend the well known fact that the external variable factors, light and temperature, are of the greatest importance in the growth of plants. Generally, the growth of plants is closely correlated with the amount of light and degree of temperature, since rising temperature and light intensity are attended, within certain limits, by increased rate of growth; outside the limits the stimulative effect is overcome by physiological disturbances in the plant. The wave length of the light to which the plants are exposed influences the growth rate and formation of chlorophyll in the plant; the most effective wave lengths are obtained from the complete sunlight or from a light source that gives a quality of light similar to sunlight.

Other Factors

Other factors that are known to be of influence in the growth of plants, but which are of minor importance in this investigation are (a) effect of seed on the growth of seedlings, /the successful growth of seedlings in nutrient solutions from which some of the essential elements had been omitted was attributed to the elements stored in the seed by Hixon (65), Kempton (81), Corson and Bakke (41), and Rotunno (108)/, (b) renewal of solutions, and (c)

volume of nutrient solutions.

That continuous aeration of the nutrient solution in which the plants were being grown increased the growth response of the plants was reported by Beals (11), Pember (102), Andrews and Beals (4), Andrews (3), Allison (2), and Clark and Shive (31); nevertheless, Loo (88) observed no difference between paddy rice, wheat, or azuki beans grown in non-aerated solutions and in solutions which were aerated for 4 minutes three times per day. Zinzadzé (140) stated that aeration for 10 to 15 minutes, three times per day was sufficient to renew the oxygen in the solutions.

Barnette (9) observed that in all cases, either the use of a large volume of nutrient solution or more frequent renewal of the nutrient solution, showed a marked advantage for the growth of plants over the use of small volumes which were renewed less frequently. This observation of Barnette's was confirmed by Breazeale (22), Hall, Brenchley, and Underwood (61), Brenchley (23), Stiles (121, 122), Hoagland (68), Tottingham and Rankin (131), Trelease and Livingston (133), Barnette and Shive (10), and Loo (88).

Growth Factors and Lemna

In a study of the influence of any single variable factor upon the growth of plants all the factors which have

been shown to have a variable effect upon plant growth must either receive consideration in the final analysis of the data obtained, or must be fixed during experimentation so that their influence may be considered as a constant.

In the experimental work of this investigation several of the factors influencing growth are fixed as nearly as possible. The species of plant used was always the same; the temperature was controlled experimentally at 25° C.; except in one phase of the work, the plants were exposed to light of constant wave length and intensity--as nearly as mazda lamps are able to furnish constant light; the time of exposure was always fixed at 14½ hours daily. The humidity, which is also a factor effecting growth, was approximately the same in all cases since the plants used, Lemna major, grow on the surface of the nutrient solution. Aeration of the solutions was not necessary because of the large surface of the solution exposed to the air. Any influence on plant growth attributed to the presence of the seed need not be considered here since Lemna major is not a seedling. The renewal of the solutions and the solution volume are constant in these experiments because equal volumes of nutrient solutions were used in all cases and the solutions renewed at regular intervals.

The factors which are varied experimentally as a part of this investigation are:--concentration of the nutrient

solution, physiological balance, phosphate-calcium ratio, reaction of the nutrient solution, and organic matter; these factors will be given consideration when an interpretation of the data is made.

METHODS

Preparation of Alkaline Humus Extract

The method employed in the preparation of the alkaline humus extracts to be used as the source of organic matter in the nutrient solutions was similar to that suggested by Burk et al. (28). The basic stock solution of potassium humate was prepared by the following procedure: eight hundred grams of a peat soil, which contained a large quantity of organic matter, was moistened with one liter of approximately normal hydrochloric acid, and allowed to stand for two hours to bring about complete solution of the calcium salts. The resulting mixture was filtered by suction and washed several times with distilled water to remove completely the dissolved salts and the excess hydrochloric acid. The residual matter was divided into four portions and each portion was placed in a three liter screw-cap shaking bottle, along with 2,250 cubic centimeters of a four per cent solution of potassium hydroxide. The resulting mixtures were then agitated by means of a mechanical shaking machine for twenty hours, after which they were allowed to stand for twenty additional hours. To the mixture of soil and dissolved humus was added an equal volume of distilled water and the resulting suspension was centrifuged for 15-20

minutes, or until the black potassium humate solution could be removed from the centrifuge bottles without disturbing the undissolved soil residue. This potassium humate solution was saved for further purification and the soil residue was discarded. To the potassium humate solution was added concentrated hydrochloric acid until the reaction was approximately pH 1.0, as indicated by the pink color produced on Orange IV indicator paper. A dark brown, almost black, precipitate resulted, which was also removed from the solution by centrifuging until the supernatant liquid could be removed from the centrifuge bottles without disturbing the residual material. To the precipitate remaining in the centrifuge tubes was added just enough ten per cent potassium hydroxide to bring about apparently complete solution; this mixture was then shaken for fifteen hours to insure more complete solution. To purify this potassium humate solution further, the solution was again precipated with hydrochloric acid and redissolved with potassium hydroxide. The resulting potassium humate solution was set aside for several months; during this time a small quantity of undissolved material accumulated at the bottom of the container. The clear black solution was decanted from this residue and was reserved for the preparation of nutrient solutions. This potassium humate solution, which contained some iron, is designated "basic stock solution of iron humate".

The quantity of dry matter in each cubic centimeter of the basic stock solution of iron humate was determined by measuring 25.0 cc. portions into tared crucibles, removing the major portion of the water by evaporation on a steam bath, and then drying to constant weight in a 110° C. oven. The results of this analysis are given in Table II.

Table II. Average dry matter in the basic stock solution of iron humate.

Volume of basic stock solution of iron humate Cc.	Total weight of crucible and dry matter Grams	Tared weight of crucible Grams	Dry weight of 25-cc. of iron humate solution Grams	Dry matter per cc. of iron humate solution Grams
25.0	22.5773	21.5073	1.0700	0.0428
25.0	22.6022	21.5328	1.0694	0.0428
25.0	29.3177	28.2504	1.0673	0.0427
25.0	23.5135	22.4412	1.0723	0.0429

The average dry matter in each cubic centimeter of the basic stock solution of iron humate is shown by Table II to be 0.0428 grams or 42.8 milligrams.

Determination of the Iron Content of the Basic Stock Solution of Iron Humate

The iron content of the basic stock solution of iron humate was determined by a method developed as a part of this investigation. The potassium humate solution used in the development was a solution prepared in a similar manner to the basic stock solution of iron humate. Two procedures are involved for determining the iron content in a solution containing organic iron, (a) the oxidation of the organic matter, and (b) the determination of the iron, usually colorimetrically. The removal of the carbon may be carried out by heating (the dry method) or by wet oxidation. The colorimetric determination most frequently involves potassium thiocyanate; comparison is made between the unknown and a standard iron solution similarly treated. The color produced by the iron and the thiocyanate fades, and fresh standard solutions must be made at frequent intervals.

Some difficulty was experienced in obtaining duplicate results from equal quantities of the iron humate when the dry oxidation method was used and followed by the potassium thiocyanate determination.

Yoe (139) suggested 7-iodo-8-hydroxyquinoline-5-sulfonic acid, $C_9H_4N(OH)I(SO_3H)$, as a reagent for the colorimetric determination of iron. The name "Ferron" has been proposed

for the indicator (60).

Yoe (131) found that this dye gave a light green color when the ferric ion was present in the concentration of about 1 part in 10 million and that a darker green was obtained when the iron concentration was increased. Ferrous iron and about 70 other ions produced no color with this dye; however, if present in large amounts, titanium, tin, and cupric ion needed removal. Yoe found that the color developed best in a solution acid to methyl orange paper and that the color was destroyed by strong acids and bases. In solutions having the correct reaction the color was stable for a considerable length of time; this avoided the necessity of preparing the fresh standards which are required by the thiocyanate method.

The advantages of this method were combined with those of Kooh and McMeekin's (82) hydrogen peroxide wet oxidation method.

For this procedure small micro-Kjeldahl flasks were made by sealing off the side arms of small Pyrex distillation flasks and calibrating them to hold 70 cc. The organic solution was oxidized by heating with concentrated sulphuric acid and adding the hydrogen peroxide drop by drop. The carbon free solution was then analyzed for iron.

Yoe (139) stated that best results were obtained with the "ferron" if the solution was made acid to methyl orange paper. Aliquots of the oxidized solution were therefore

treated with 0.4N potassium hydroxide until just acid to the paper, or methyl orange was used as an internal indicator, potassium hydroxide being added until a faint pink color was obtained; the same amount of potassium hydroxide was then added to a colorless second aliquot and the iron determined in this. In both cases the results were somewhat unsatisfactory. There was a large color change in the green produced by the reagent when a slight excess of either potassium hydroxide or acid was present.

To determine the influence of reaction on the color, a standard solution was prepared from recrystallized ferrous ammonium sulfate; the ferrous ion was oxidized with bromine to the ferric ion and the excess bromine was removed by boiling for several minutes. Various quantities of potassium hydroxide and sulfuric acid were added to equal aliquot portions of this standard solution and the color developed by adding "ferron". A wide variation of colors resulted. Some solutions were colorless when they contained potassium hydroxide; others were blue-green when they were more acid; however, in several solutions, which contained different quantities of acid and base, the color was the same. The pH values for all these solutions were determined, and it was observed that the group of solutions having the same colors fell within the reaction range of pH 2.7 to 3.2. Other concentrations of iron in standard solutions were

tested in the same reaction range and they also gave a stable color. In making dilutions, therefore, conductivity water which was acidified to pH 2.7 to 3.2 was used. It was found that the standard iron solution deteriorated very slowly after three days and did not give a color with "ferron" equal to that in a freshly prepared solution; however, when the color was developed with the dye in the ferric iron standard solution, it was found to match with the color in freshly prepared solutions of the same concentration for a period of 25 days.

The treatment of the unknown solutions was modified to conform with this finding. After digesting the organic matter with sulfuric acid and hydrogen peroxide and making up to 70 cc., a 10 cc. aliquot was removed, and 0.1N potassium hydroxide added until a faint blue color was produced, when diluted to 40 cc. with distilled water, with 2 drops of bromophenol blue as internal indicator; 0.8 cc. of 0.1N sulfuric acid brought this to the range 2.7 to 3.2. The amount of sulfuric acid subtracted from the potassium hydroxide left the net amount of potassium hydroxide required. A fresh 25 cc. aliquot of the oxidized solution was then used, with two and one-half times the amount of potassium hydroxide found for the 10 cc. sample, and brought exactly to 100 cc. for nesslerization. With the use of a glass electrode to adjust the pH, the indicator is not necessary,

but the bromophenol blue method is more rapid.

Table III gives the results for an extract of soil with potassium hydroxide--the potassium humate solution. The alkali extract of soil contained a small amount of silicate, but this did not interfere, as shown in Table III. To check this possible silicate interference further, a run was made using the standard iron solution, but adding 0.1 to 1 mg. of silica as sodium metasilicate. The iron was recovered completely at each silicate level.

The satisfactory results from the hydrogen peroxide oxidation and the "ferron" analysis on the known solutions allowed the methods to be used on the stock iron humate. Fifty cc. of the basic stock solution of iron humate were diluted to five hundred cc., and from the resulting solution twenty-five cc., aliquot portions (equal to 2.5 cc. of the basic stock solution of iron humate) were transferred to the five micro-digestion flasks; the organic matter was destroyed by hydrogen peroxide and the volume of the solution in each flask was made up to 70 cc. Twenty-five cc. aliquot portions were removed from each flask to 100 cc. Nessler tubes and adjusted to pH 2.7-3.2 by adding a calculated amount of 0.1 normal potassium hydroxide. The calculated amount of potassium hydroxide added was determined by titrating another 25 cc. portion of each solution with the same base until the color was faintly blue to bromo-phenol

Table III. Iron in potassium humate.

Amounts oxidized		pH*		Total iron present				
Potassium humate Cc.	Standard Fe, 2 mg. per liter Cc.	0.1N KOH to bring 10-cc. aliquot to pH 2.75-3.2 Cc.	0.1N KOH for 25-cc. aliquot (calc'd.) Cc.	Standard Fe (2 mg. per liter) to match 25-cc. aliquot** Cc.	Fe in 70-cc. oxidized solution Mg.	Blank Mg.	Fe in sample Mg.	Fe in humate solution Mg./cc.
0	50.0	11.7	29.2	19.0	0.106	0.006	0.100	....
0	50.0	12.7	31.7	19.0	0.106	0.006	0.100	....
0	50.0	13.5	33.7	19.0	0.106	0.006	0.100	....
50.0	0	10.6	26.5	7.0	0.039	0.006	0.033	0.0007
50.0	0	9.3	23.2	7.0	0.039	0.006	0.033	0.0007
50.0	0	9.8	24.5	7.0	0.039	0.006	0.033	0.0007
25.0	25.0	11.1	27.7	13.0	0.073	0.006	0.067	0.0007
25.0	25.0	11.4	28.5	13.0	0.073	0.006	0.067	0.0007
25.0	25.0	12.3	30.7	13.0	0.073	0.006	0.067	0.0007

*Potassium hydroxide added until faint blue to bromophenol blue when diluted to 40 cc. 0.8 cc. 0.1N sulfuric acid brought this to pH 2.75 to 3.2. The difference, multiplied by 2.5, gave the net cc. of potassium hydroxide to be added to the 25-cc. aliquot before making up to 100 cc. for the Nessler tube.

**Diluted to 100 cc. at pH 2.75 to 3.2; 1 cc. of color reagent, 0.2 per cent.

blue; 2.0 cc. was subtracted from the observed value in order to reach the 2.7-3.2 range as indicated in the preliminary experiments.

These solutions were compared colorimetrically to a series of freshly prepared standards. The results of the analysis are given in Table IV.

From Table IV the average amount of iron per cubic centimeter of basic stock solution of iron humate is 0.0426 mg. In Table I each cubic centimeter of the basic stock solution was shown to contain 42.8 mg. of dry matter, thus for each milligram of dry matter there was approximately 0.001 mg. of iron.

Technique for Lemna Major

In this series of experiments "growth" is used as synonomous with rate of reproduction; as a general rule the size of the plants correlates well with the rate of reproduction and the measurements were made here on the basis of the latter. Lemna major reproduces by budding; the mother and daughter fronds separate and both bud again. When conditions suitable for growth are kept constant the rate of reproduction, K, may be calculated by using the equation derived by Clark (35), $\log_{10}N - \log_{10}N_o = K(t - t_o)$, in which N is the number of fronds at any time, t. The rate of reproduction is determined graphically by plotting $\log_{10}N$

Table IV. Iron in basic stock solution of iron humate.

Sample No.	Aliquot (50cc.-500cc.) diluted iron humate Cc.	Equivalent to basic stock solution of iron humate Cc.	Diluted in micro-digestion flasks to Cc.	Standard Fe (2 mg. per liter) to match 25 cc. aliquot Cc.	Fe in 70cc. oxidized solution Mg.	Blank Mg.	Fe in 2.5 cc. of basic stock solution of iron humate Mg.	Fe in Basic stock solution of iron humate Mg./cc.
1	25.0	2.5	70.0	24.0	0.1344	0.0280	0.1064	0.0426
2	25.0	2.5	70.0	24.0	0.1344	0.0280	0.1064	0.0426
3	25.0	2.5	70.0	24.0	0.1344	0.0280	0.1064	0.0426
4	25.0	2.5	70.0	24.0	0.1344	0.0280	0.1064	0.0426
5	25.0	2.5	70.0	23.0	0.1288	0.0280	0.1008	0.0403
$Blank_1$	0.0	0.0	70.0	5.0	0.0280	0.0280	----	----
$Blank_2$	0.0	0.0	70.0	5.0	0.0280	0.0280	----	----

against the time in days, t; the slope of the resulting curve represents the rate of reproduction, K.

The plants used in these experiments were free from microorganisms (40) and were grown in sterile nutrient solutions. The nutrient solutions used were of the same composition as that formulated by Clark (36) and found by him to give optimum reproduction of Lemna major at pH 4.7 to 4.9. These solutions contained 0.4 millimols of calcium per liter added as mono-calcium phosphate, 8 millimols of potassium as potassium nitrate, 1 millimol of magnesium as magnesium sulfate, 0.00046 millimols of manganese as manganous chloride, and 0.01 millimols, (0.062 mg.), of iron as ferric chloride. This solution will be designated as "Clark's solution".

In all cases the concentration of the elements was as given above, except that of the iron and potassium. The exact amount of potassium in the cultures was not determined; 8 millimols of potassium was added as potassium nitrate and small variable quantities were added contained in the iron humate solution. The iron concentration was varied in each experiment and the amount will be noted in each case.

In all experiments the plants were grown in 100 cc. of the nutrient solution contained in a 250 cc. Erlenmeyer flask. The plants were grown at 25° C. in the constant light and temperature apparatus described by Clark (38),

the light being furnished by four 300 watt mazda bulbs for 14½ hours daily except where stated otherwise. The intensity of the light was measured by a Weston Illumination Meter and was found to be 200 foot-candles at the surface of the plants. The sterile plants were transferred to freshly prepared cultures twice weekly, except where otherwise designated; all transfers were made in the sterile transfer chamber (38). All cultures were checked periodically for contamination by inoculating a sterile nutrient-agar slant with one of the plants. When there was a contamination, which was indicated by bacterial growth on the agar within a few days after inoculation, a new culture was started..

EXPERIMENTAL

Influence of Iron Humates on Growth and Chlorosis of Lemna

Experiment 1. The influence of various quantities of iron humate on the growth of sterile Lemna major at pH 4.8 and the intensity and quality of light as growth factors.

Duplicate nutrient solutions were prepared which contained various quantities of iron humate. Cultures 1 and 2 were Clark's solutions and were used as controls for the other cultures. Cultures 3 and 4 contained all of the salts in the concentration of Clark's solution and enough iron humate to give 0.064 mg. of iron and 64.2 mg. of dry matter, thus the total iron of these cultures was 0.126 mg. per 100 cc. culture. Cultures 5 and 6 contained all the salts in the concentration of Clark's solution except iron as ferric chloride, which was ommitted, but enough iron humate to give 0.064 mg. of iron and 64.2 mg. of dry matter; these cultures, therefore, contained 0.064 mg. of iron per 100 cc. of culture. All of these cultures were adjusted to pH 4.8, diluted to 100 cc., and sterilized by autoclaving.

Eight or nine normally developed, sterile, Lemna major fronds were transferred to each culture. Cultures 1, 3, and 5 were grown in the constant light and temperature chamber

previously described, and which will, henceforth, be abbreviated by C.L.T.A. These cultures were continued for seven weeks during which time the plants were transferred to freshly prepared solutions once each week. At the time of each transfer the number of fronds in each flask was counted and eight or nine fronds were transferred to each of the new solutions. The rate of reproduction, K, was determined graphically in Figure 1 by plotting $\log_{10}N$ against the time in days, t.

Cultures 2, 4, and 6, were grown in a newly constructed constant light and temperature chamber which used 10 red neon tubes as the source of light and was held at a temperature of 25° C. These light tubes were 33 inches from the plant surfaces, at which point the intensity of the light was 100 foot-candles. The intensity was measured by a Weston Illumination Meter, Model 603, which had been standardized against the light produced by a tungsten filament at 3000° Kelvin. This neon light source will henceforth be termed R.N.C. These three cultures were grown under the conditions of R.N.C. for five weeks with the same technique as used in the cultures grown under C.L.T.A.; the reproduction curves for the plants in this series are represented by portions A to B of curves 2, 4, and 6 in Figure 1.

At the end of five weeks the light source was changed in the R.N.C. by removing four of the red tubes, and substi-

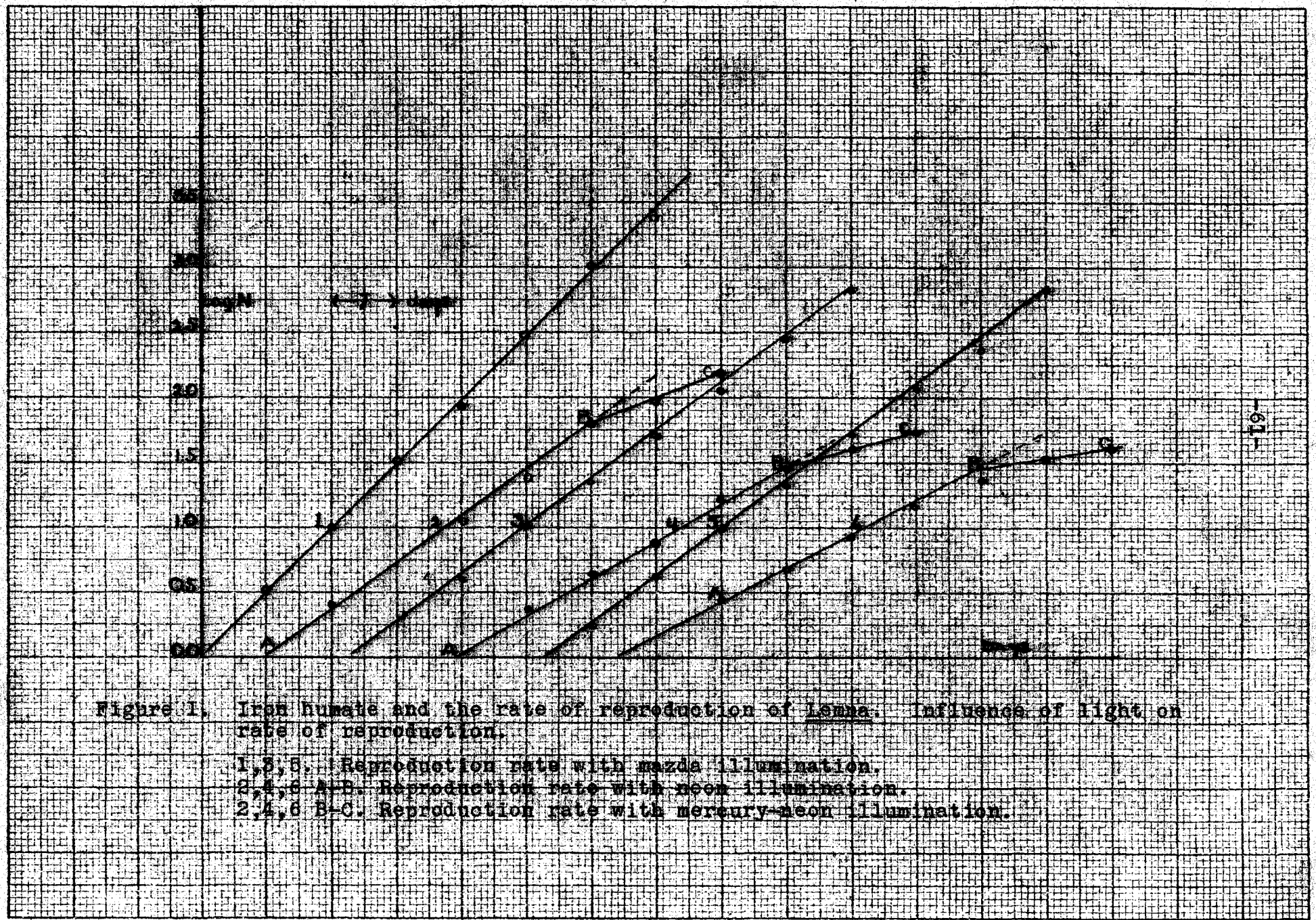

Figure 1. Iron humate and the rate of reproduction of Lemna. Influence of light on rate of reproduction.

1,3,5. Reproduction rate with mazda illumination.
2,4,6 A-B. Reproduction rate with neon illumination.
2,4,6 B-C. Reproduction rate with mercury-neon illumination.

tuting for them four blue mercury-neon tubes. The intensity of the light at the surface of the plants was found to be 60 foot candles. This light source will be designated R.B.N.C. Cultures 2, 4, and 6 were continued for two additional weeks under R.B.N.C. and the reproductions are represented by the portions B to C of curves 2, 4, and 6 in Figure 1.

The influence of various quantities of iron humate on the rate of growth of Lemna major at pH 4.8 is shown by Table V. The effect of varying the light quality and light intensity upon the rate of growth is also recorded in Table V.

Of the cultures under mazda illumination, Clark's solution, containing no organic matter, gave much better growth than did either of the cultures containing iron humate. The cultures containing iron humate and ferric chloride plus iron humate gave equal responses, but the growth was much less than in Clark's solution. The same relative response was made by the plants illuminated with red or with combined red and blue neon light, but the actual growth rate under the red-neon illumination was less than under mazda, and the blue in turn less than the red.

It has been reported (48) that ultra-violet light of wave lengths less than 313 millimicrons is injurious to the growth of some green plants. Therefore, since the plant

Table V. Influence of iron humate on growth of Lemna at pH 4.8. Effect of light on rate of growth.

Culture No.	Fe as ferric chloride Mg./L.	Fe as iron humate Mg./L.	Total iron Mg./L.	Total iron Millimols per Liter	Total dry matter as iron humate Mg./L.	K x 100 from Fig. 1 C.L.T.A.* 200 f.c.	K x 100 from Fig. 1 A to B R.N.C.** 100 f.c.	K x 100 from Fig.1 B to C*** 60 f.c.
1	0.62	0.00	0.62	0.01	00.00	7.0	---	---
2	0.62	0.00	0.62	0.01	00.00	---	5.1	2.8
3	0.62	0.64	1.26	0.02	642.0	5.2		
4	0.62	0.64	1.26	0.02	642.0	---	4.1	1.7
5	0.00	0.64	0.64	0.01	642.0	5.2		
6	0.00	0.64	0.64	0.01	642.0	---	3.7	1.3

* C.L.T.A. Plants exposed for 14½ hours daily to 200 foot-candles from mazda lamps. Temperature 25° C.

** R.N.C. Plants exposed for 14½ hours daily to red neon lights with 100 foot candles intensity. Temperature 25° C.

*** R.B.N.C. Plants exposed for 14½ hours daily to 6 red and 4 blue neon lights with 60 f.c. intensity. Temperature 25° C.

growth was greatly reduced after the blue neon tubes were used to replace part of the red tubes, it was desirable to find what wave lengths of light were being furnished by the blue-neon tubes. A spectrum-photograph was taken of the light produced by one of the blue tubes; it was found to contain four lines at the 302.1 millimicron group and one line at 296.7 millimicrons. No lines were observed below 296.7 millimicrons and all those below 313.2 were barely discernable on one hour exposure*. Part of the depression might, therefore, be attributed to the waves below 313 millimicrons.

Experiment 2. The influence of light intensity upon the growth of Lemna major.

In Experiment 1 it was observed that the growth of Lemna major was greatly inhibited when the plants were exposed to the combined red-blue neon illumination, (R.B.N. C.). It was also noted that the intensity of this light at the plant surface was far less than that in the C.L.T.A. with which its efficiency for plant growth was compared.

In the following experiment five sterile cultures of Lemna major were grown in Clark's solution at pH 4.8, at various distances from the red-blue neon tubes, for the

*The author wishes to thank Dr. H. A. Wilhelm for his kindness in taking the spectrum-photograph.

purpose of determining the effect of light intensity upon growth. One culture was grown in C.L.T.A. with mazda illumination as a control culture. The plants were grown for a period of six weeks; they were transferred to freshly prepared solutions twice each week for the first three weeks; during the last three weeks they were transferred to new solutions only once each week. The growth rate at each level was determined graphically in Figure 2 by the method described previously. The intensities of the light at each distance from the tubes were determined by the Weston Illumination Meter; they are recorded along with the various distances in Table VI.

Table VI and Figure 2 show the influence of light of various intensities upon the growth rate of Lemna major. Culture 1 of Table VI is represented graphically by curve 1 of Figure 2, and culture 2 by curve 2.

When the rates of growth at various intensities of light from the same source (cultures 2, 3, 4, 5, and 6) are compared, it is apparent that the increased intensities give increased growth rates; however, if the data of cultures 1 and 2 are compared it is seen that the rate of growth under neon illumination at 100 foot-candles intensity is equal to that of the control culture grown under 200 foot candles from mazda. Apparently, for equal foot-candle power the neon-mercury light is more efficient, but the accuracy of

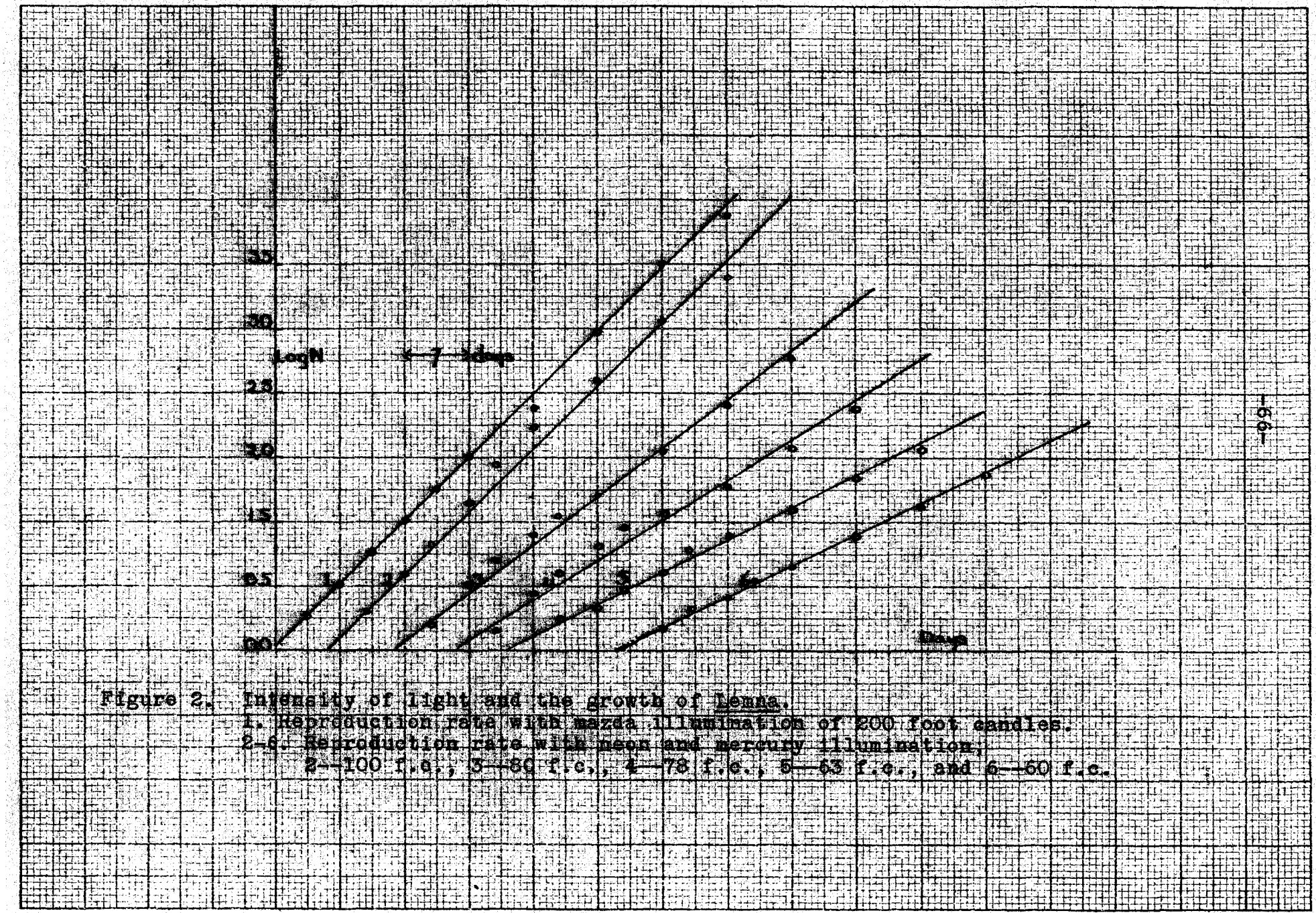

Figure 2. Intensity of light and the growth of Lemna.
1. Reproduction rate with mazda illumination of 200 foot candles.
2-6. Reproduction rate with neon and mercury illumination;
2--100 f.c., 3--80 f.c., 4--78 f.c., 5--63 f.c., and 6--60 f.c.

Table VI. Intensity of light and the growth of Lemna major.

Culture No.	Source of light	Distance from light source inches	Intensity of light Foot-candles	K x 100 from Fig. 2
1	C.L.T.A.*	21.5	200	7.0
2	R.B.N.C.**	6.625	100	7.0
3	"	13.25	80	5.3
4	"	19.875	78	4.5
5	"	26.5	63	3.7
6	"	33	60	3.5

* C.L.T.A. Constant light and temperature apparatus. Illumination with mazda lamps. Temperature 25° C.
** R.B.N.C. Red and Blue neon chamber. Illumination with neon and mercury lamps. Temperature 25° C.

the Weston Illumination Meter for measuring light intensity from neon and mercury lights in not established.

Experiment III. The influence of iron humate upon the growth and chlorosis of Lemna major at various reactions.

Olsen (97, 98) added alkaline humus extract to nutrient solutions containing ferric chloride as the source of iron; he observed a depression in the growth of Lemna major at pH 4.0-5.0 and a stimulation of the growth at reactions more alkaline than pH 5.0; the maximum growth was obtained at pH 7.0. In cultures containing ferric chloride but no humus extract the optimum growth was obtained at pH 4.0 to 5.0, with poor growth and very bad chlorosis at pH 6.0-7.0; however, if ferric citrate was used instead of ferric chloride, good growth was obtained in all reactions between pH 4.0 and pH 8.0, with maximum growth at pH 7.0. No added stimulation of growth at reactions more basic than pH 6.0 was observed when humus extracts were added to the cultures containing ferric citrate.

It has been shown in Experiment 1 that quantities of iron humate depressed the growth of Lemna major in Clark's sterile solution at pH 4.8. To determine the influence of various quantities of iron humate upon the growth of Lemna major at various reactions in sterile solutions, a series of 38 cultures of modified Clark's solution was prepared.

In all of these cultures, except numbers 28 to 34 inclusive, the total iron concentration was 0.01 millimols per liter; the concentration of iron in cultures 32, 33, and 34 was 0.02 millimols per liter and in 28 to 31 was 0.002 millimols per liter. The amount of iron humate in this series of cultures was varied to give different total iron—iron humate proportions. As the amount of iron humate was reduced it became necessary to add ferric chloride to bring the total concentration of iron up to 0.01 millimols per liter of nutrient solution.

Eight cultures containing ferric citrate and three cultures containing ferric chloride were also prepared. In all of these solutions the amount of iron was 0.01 millimols per liter. These cultures served as control cultures and received the same treatment as the humate cultures.

The culture media were prepared from stock solutions and adjusted to the desired pH with previously determined quantities of dilute potassium hydroxide or hydrochloric acid. The pH's of the solutions are recorded in Table VII. Sterile technique was used throughout the experiment, and all plants were exposed to the C.L.T.A. for 14½ hours daily. The experiment was continued for five to six weeks, and the plants were transferred to freshly prepared solutions twice weekly at regular intervals. At the time of transfer the fronds were counted and from 15 to 20 fronds transferred to

the new solutions; this number was kept fairly constant to insure the same relative response in all cultures.

The influence of various quantities of iron humate upon the rate of reproduction of Lemna major in sterile nutrient solutions of various reactions is shown graphically in Figure 3. The cultures in which the plants died within a short time after initiation of the experiment were omitted from the graph as sufficient data was not obtained to plot the growth curves.

In this series the plants developed abnormally in certain of the cultures, giving fronds which were either larger or smaller than the average fronds produced in sterile nutrient solutions. In these cases the conditions of the plants are recorded along with the reproduction rate, K, in Table VII.

Table VII shows the effect of variable quantities of organic matter, the iron humate, upon the health and rate of reproduction of the Lemna in nutrient solutions which have either a fixed concentration of iron or a variable concentration of iron. This table also shows the influence of reaction upon the availability of iron in solutions containing ferric chloride, ferric citrate, and iron humate.

The data in Table VII showed that the growth of Lemna major was prohibited in all solutions at pH 3.5 or 4.0; all of these cultures died within a few days after inoculation. The maximum growth in the solutions containing only ferric

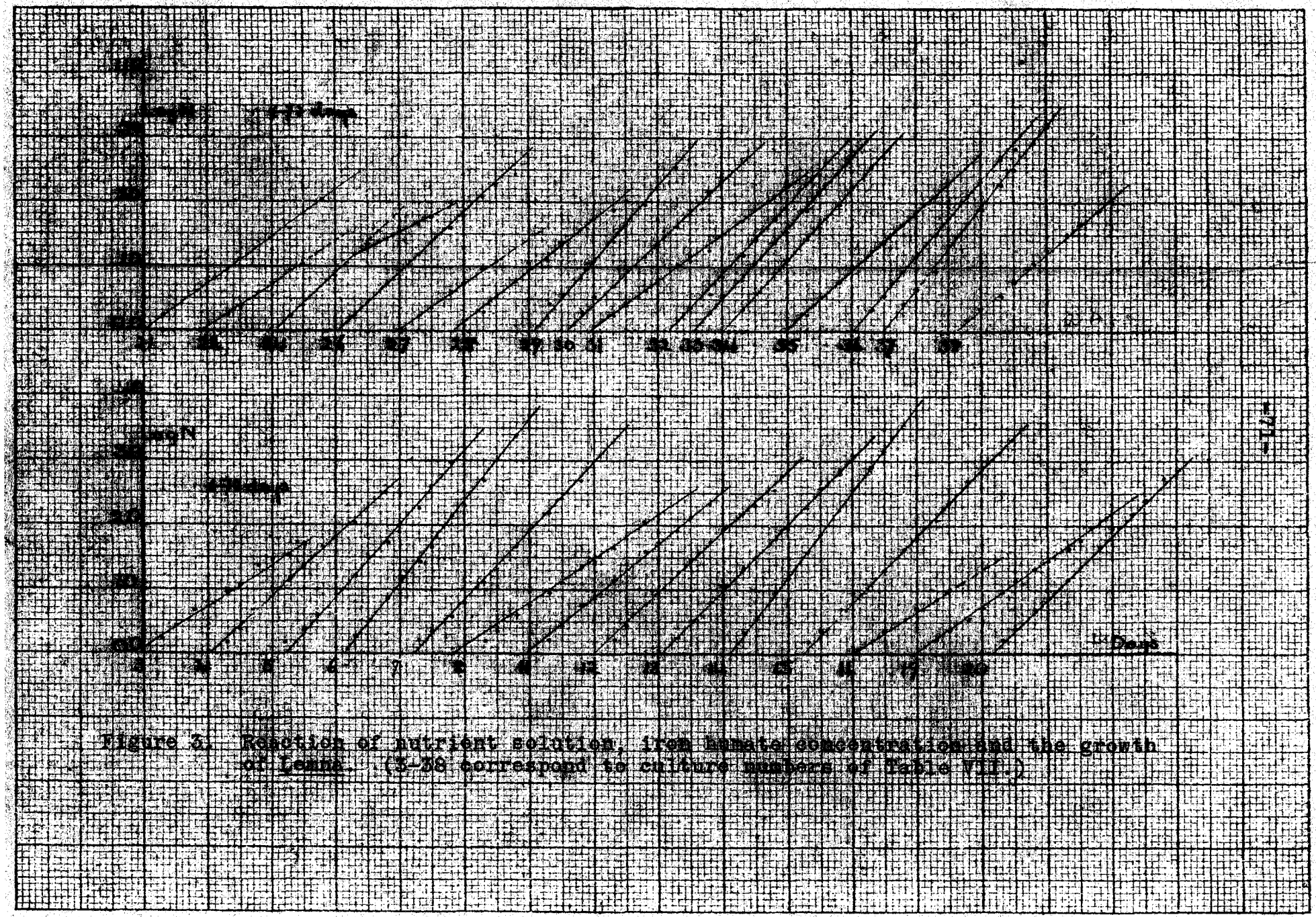

Figure 3. Reaction of nutrient solution, iron humate concentration and the growth of Lemna. (3-38 correspond to culture numbers of Table VII.)

Table VII. Reaction of Nutrient Solution, Iron Humate Concentration, and the Growth of *Lemna major*.

Culture No.	Initial pH of solution*	Source of iron	Total Fe in one liter of culture Millimols.	Total Fe in one liter of culture Mg.	Total iron humate** Mg./L.	Total iron-iron humate ratio	Kx100 from fig. 3.	Condition of plants.
1	3.5	I.H.**	0.010	0.62	622	0.001	---	died within 3 days
2	4.0	"	0.010	0.62	622	0.001	--	died within 3 days
3	4.5	"	0.010	0.62	622	0.001	4.5	developed into very small, dark green, plants
4	5.0	"	0.010	0.62	622	0.001	6.9	" " " "
5	6.0	"	0.010	0.62	622	0.001	8.3	normally green, small plants
6	7.0	"	0.010	0.62	622	0.001	9.0	" " , average size plants
7	8.0	"	0.010	0.62	622	0.001	7.6	light green, average size
8	9.0	"	0.010	0.62	622	0.001	5.2	very pale green, " " badly clumped
9	3.5	I.H.+ $FeCl_3$	0.010	0.62	311	0.002	---	died within 3 days
10	4.0	"	0.010	0.62	311	0.002	--	" " " "
11	4.5	"	0.010	0.62	311	0.002	5.9	dark green, very small
12	5.0	"	0.010	0.62	311	0.002	6.8	" " , small but somewhat larger than #11

Table VII. (continued).

Culture No.	Initial pH of solution*	Source of iron	Total Fe in one liter of culture Millimols.	Total Fe in one liter of culture Mg.	Total iron humate** Mg./L.	Total iron-iron humate ratio	Kx100 from fig. 3.	Condition of plants.
13	6.0	I.H.+ $FeCl_3$	0.010	0.62	311	0.002	7.4	normally green, average size
14	7.0	"	0.010	0.62	311	0.002	9.6	" " " " , very thin leaves
15	8.0	"	0.010	0.62	311	0.002	7.6	pale green, average size, very thin leaves
16	9.0	"	0.010	0.62	311	0.002	5.0	chlorotic within 2 weeks
17	3.5	Ferric Citrate	0.010	0.62	---	---	---	died in 3 days
18	4.0	"	0.010	0.62	---	---	---	" " " "
19	4.5	"	0.010	0.62	---	---	5.1	very dark green, very large, badly clumped plants
20	5.0	"	0.010	0.62	---	---	7.2	normally green, average size
21	6.0	"	0.010	0.62	---	---	5.2	" " , very large thin, and badly clumped
22	7.0	"	0.010	0.62	---	---	4.7	chlorotic within 11 days
23	8.0	"	0.010	0.62	---	---	---	chlorotic in 7 days, growth very poor
24	9.0	"	0.010	0.62	---	---	6.2-3.5	good growth for 3 weeks with very poor thereafter

Table VII. (continued).

Culture No.	Initial pH of solution*	Source of iron	Total Fe in one liter of culture Millimols.	Total Fe in one liter of culture Mg.	Total iron humate** Mg./L.	Total iron-iron humate ratio	Kx100 from fig. 3.	Condition of plants.
25	4.0	$FeCl_3$	0.010	0.62	---	---	---	died within 7 days
26	4.8	"	0.010	0.62	---	---	7.0	normally green, good growth
27	6.0	"	0.010	0.62	---	---	5.0	chlorotic within 7 days
28	5.0	I.H.	0.002	0.125	125.0	0.001	5.8	very small, normally green
29	6.0	"	0.002	0.125	125.0	0.001	8.2	" " " " , very thin leaves
30	7.0	"	0.002	0.125	125.0	0.001	6.7	average size, pale green, very thin leaves
31	8.0	"	0.002	0.125	125.0	0.001	5.2	slightly chlorotic, average size, very thin leaves
32	6.0	"	0.020	1.24	1244.0	0.001	7.7	very small plant, dark green, thin leaves
33	7.0	"	0.020	1.24	1244.0	0.001	7.7	" " " , " " " "
34	8.0	"	0.020	1.24	1244.0	0.001	7.9	" " " , slightly yellow, thin leaves
35	5.0	I.H.+ $FeCl_3$	0.010	0.62	125.0	0.005	6.6	normally green, average size

Table VII. (continued).

Culture No.	Initial pH of solution*	Source of iron	Total Fe in one liter of culture Millimols.	Total Fe in one liter of culture Mg.	Total iron humate** Mg./L.	Total iron-iron humate ratio	Kx100 from fig. 3.	Condition of plants
36	6.0	I.H.+ $FeCl_3$	0.010	0.62	125.0	0.005	8.3	normally green, average size, thin leaves
37	7.0	"	0.010	0.62	125.0	0.005	9.2	pale green, average size, thin leaves
38	8.0	"	0.010	0.62	125.0	0.005	6.2	slightly yellow, average size, thin leaves

* All pH's were determined by the glass electrode method. The electrode was calibrated against standard buffer solutions of which the pH had been determined by the quinhydrone method.

** The abbreviation I.H. means basic stock solution of iron humate which has been described previously.

** Total iron humate is synonymous with dry matter; the dry matter was determined by evaporating a definite volume of the solution of iron humate to dryness at 110° C.

chloride was obtained at pH 4.8 as shown by culture 26, and in culture 27 at pH 6.0 the growth was greatly inhibited and chlorosis developed within 7 days. Cultures 17-24 showed that the maximum growth for cultures containing ferric citrate was at pH 5.0, with growth diminishing as the reaction increased; chlorosis was quite evident in the cultures at pH 7.0-8.0. In culture 24, containing ferric citrate at pH 9.0, the plants developed normally for about three weeks, after which the growth was poor and chlorosis was pronounced.

The data of Table VII concerning the influence of various quantities of iron humate at different reactions can be seen more clearly by treating the results graphically. A graphical representation is given in Figure 4, in which the rate of reproduction, K, is plotted against the initial pH of the nutrient solutions.

In cultures 1 to 8 inclusive the source of iron was iron humate in a concentration which gave 0.62 mg. of iron and 622 mg. of organic residue per liter of culture. The plants in cultures 1 and 2 at pH 3.5 and 4.0 died soon after the start of the experiment, while those in cultures 3 to 8 developed well and reproduced in fairly constant rate for a period of six weeks. The plants which developed in cultures with the most acid reactions--pH 4.5-5.0--were very small and were much darker green than the plants in the more alkaline solutions. The plants at pH 6.0 and 7.0 were of the

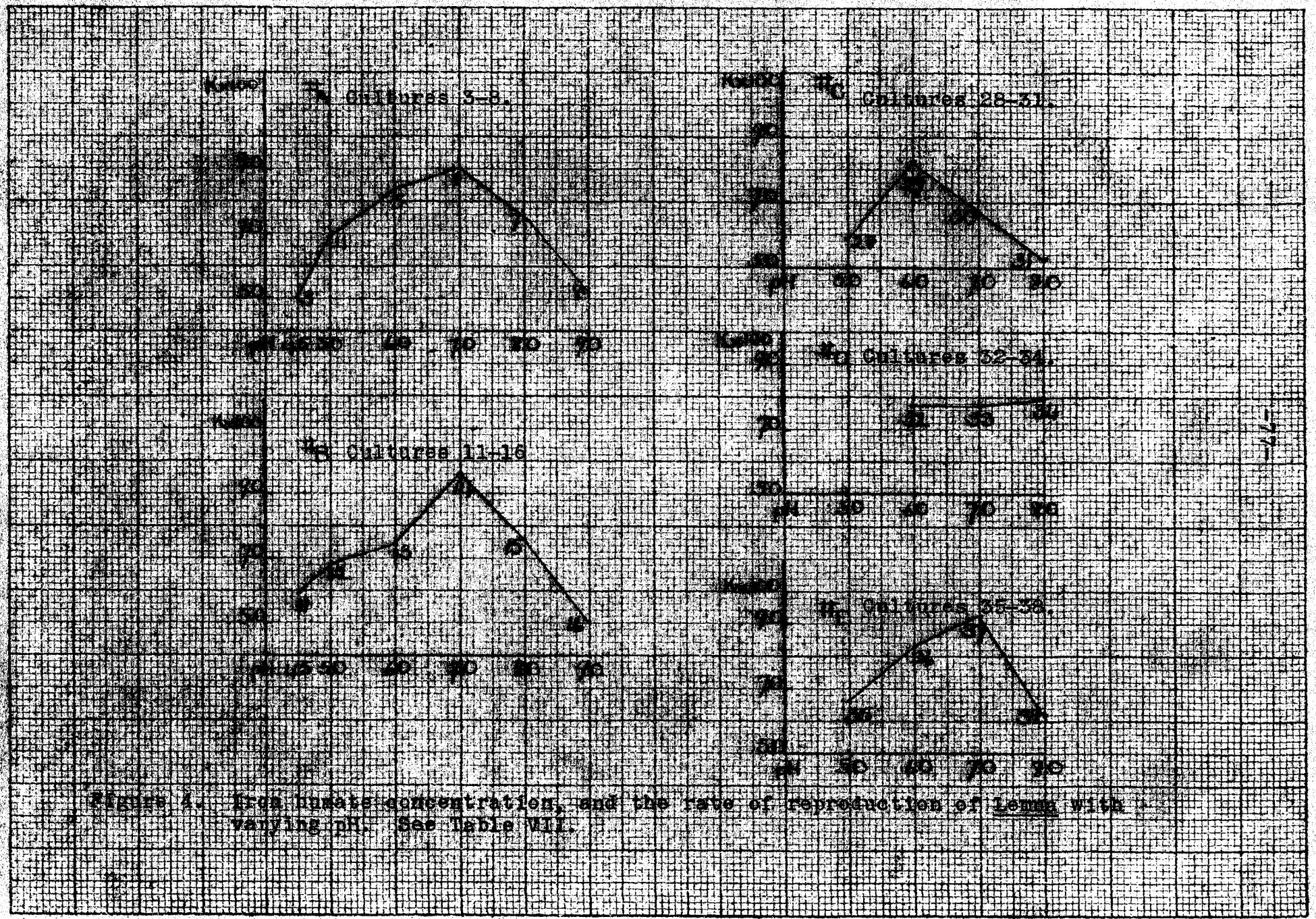

Figure 4. Iron humate concentration, and the rate of reproduction of Lemna with varying pH. See Table VII.

average green color of Lemna grown under sterile conditions; the frond size of the culture at pH 7.0 was average while that for the culture at pH 6.0 was somewhat smaller than average. Cultures 7 and 8, at pH 8 and 9 respectively, produced Lemna of average size but the plants were a very light color; they were almost chlorotic at pH 9.0 although the chlorophyll seemed to be evenly distributed in the fronds. Part #A of Figure 4 shows the change in rate of reproduction, K, with variation of the initial reaction of the medium. The rate of reproduction increases progressively from pH 4.5 to 7.0 and then decreases progressively as the pH increases.

Cultures 9 to 16 represent a series in which the organic matter, iron humate, was reduced to one half the amount of the previous series (311 mg. per liter) and the iron concentration was made up by adding ferric chloride. The plants in cultures having a pH of 3.5 and 4.0 died within three days after the experiment was started, and all others reproduced at a constant rate. Again in this series the plants became small and dark green at pH 4.5-5.0. At pH 6.0 and 7.0 the plants were of normal size and color; however, at pH 7.0 the fronds were very thin as compared to the usual thick, velvety fronds produced in sterile cultures. In culture 15, at pH 8.0, the plants were also very thin and had a slight tinge of chlorosis while those in culture 16 at pH 9.0 became badly chlorotic within two weeks, although they did

continue to reproduce at a fairly constant rate, as is shown by curve 16 of Figure 3. In part #B of Figure 4 it was shown that the rate of reproduction in this series also increases up to a value of pH 7.0, and then decreases as the reaction becomes more alkaline. The rate of reproduction at pH 7.0 is somewhat greater than in the previous series.

When the iron humate was reduced to one fifth the amount present in the first series, 125 mg. per liter, thereby causing a similar decrease in the iron concentration to a value of 0.125 mg. per liter of solution, there was a shift in the optimum reaction for reproduction from pH 7.0 to pH 6.0 as is shown by part #C of Figure 4. The plants in cultures 28 and 29 at pH 5.0 and 6.0 were very small but were normally green; at pH 5.0 they were normal in appearance, while at pH 6.0 very thin, highly ridged fronds resulted. At pH's 7.0 and 8.0 the plants produced very thin average sized fronds which were pale green at the neutral reaction and slightly chlorotic at pH 8.0. Although the rate of reproduction in the culture at pH 6.0 was greater than at pH 7.0, the actual growth if measured by dry weight probably would have been the same, since the plants at pH 6.0 were very much smaller than those at pH 7.0.

In cultures 32, 33, and 34 the quantity of iron and organic matter in each culture was increased to double the amount in the first series. This gave an iron concentration

of 1.24 mg. per liter and 1244 mg. of iron humate. In these cultures (pH 6.0, 7.0, and 8.0) the plants were smaller than in any series attempted, and developed into very thin highly ridged fronds which were very dark green at pH's 6.0 and 7.0 and were slightly chlorotic at pH 8.0. In part #D of Figure 4 it will be noticed that the rate of reproduction was practically identical at all reactions.

Cultures 35 to 38 represent a series in which the iron humate was reduced to 125 mg. per liter of nutrient solution (one fifth of the first series of this experiment), and the iron content was kept at 0.62 mg. per liter by adding ferric chloride; the reactions of these solutions were adjusted to 5.0, 6.0, 7.0, and 8.0. The rate of reproduction, K, in this series was shown to have an optimum at pH 7.0, in part #E of Figure 4, with the rate decreasing as the reaction became either more acid or alkaline. In this series the rate of reproduction, K, is a fairly good indicator of the growth rate since the size of the plants at all reactions was about equal. The plants in the solutions with the lowest reaction (pH 6.0) were normally green; the depth of color decreased as the alkalinity of the solutions increased until at pH 8.0 the plants were slightly chlorotic.

From the data of Table VII and Figures 3 and 4 it was concluded that the optimum growth of Lemna was at pH 4.5 to 5.0 for the ferric chloride and ferric citrate series, and

that growth decreased and chlorosis increased as the reaction became more alkaline. In the series containing various quantities of iron humate, the optimum growth was at pH 7.0 when the concentration of the iron was 0.62 milligrams per liter and the ratio of the total iron to the iron humate fell within the range of 0.001-0.005; the optimum response was obtained at a ratio of 0.002. When the iron concentration was reduced to 0.125 milligrams per liter and the iron-iron humate ratio was 0.001, optimum reproduction was obtained at pH 6.0, but, when the iron was increased to 1.25 mg. with the same iron-iron humate ratio no noticeable difference was observed in the reproduction rate at pH's 6.0, 7.0, or 8.0. In all the cultures containing humates of iron, some chlorosis developed when the reaction became more alkaline than pH 7.0; this condition was more evident in the cultures having the lowest quantity of iron. The growth at pH 8.0 decreased progressively as the amount of organic matter decreased; the maximum growth was obtained in the culture containing the largest quantity of iron and organic matter.

By comparing the results of these experiments in which various forms and amounts of iron were used, it was observed that ferric chloride and ferric citrate produced the best growth at pH 4.5 to 5.0, while cultures containing iron humate gave the best response at pH 6.0 to 7.0--the maximum growth rates in the iron humate series were greater in every case than in the ferric chloride or citrate series. Further, the growth of Lemna

in sterile nutrient solutions is depressed by the organic matter at pH 4.5 to 5.0, and stimulated by it in cultures of more alkaline reactions. The organic matter also aids in better plant development by preventing chlorosis at reactions of pH 6.0-7.0.

Influence of Phosphate-Calcium Ratio on Growth and Chlorosis in Lemna

It was shown in the previous experiments that organic matter--the iron humate--was effective in preventing chlorosis and stimulating growth of Lemna in cultures of Clark's solution at pH 7.0, and that chlorosis soon developed in cultures containing no organic matter when the reaction was pH 6.0 or above. The same observation was made by Olsen (98) with Lemna in a modified Knop's solution, but he reported also that, with the reaction at pH 8.0, the plants were again normally green and gave good growth in solutions containing no organic matter; maximum chlorosis appeared at pH 6.0-7.0. This unusual result at the alkaline reaction was attributed to the precipitation of the calcium phosphate and the consequent lower phosphate concentration in the solution at pH 8.0, as compared to solutions at pH 6.0-7.0 where chlorosis was most severe. By reducing the phosphate concentration of Knop's solution to one fifth the usual quantity Olsen was able to grow maize plants for 18 days without chlorosis

developing at pH 6.0 to 7.0; maximum growth was obtained at pH 7.0.

From Olsen's results it seemed that plants would grow normally without organic matter at pH values from 6.0 to 8.0 provided the phosphate-calcium ratio was kept within definite limits. An attempt was therefore made to establish these limits for Lemna in a number of sterile solutions.

Experiment 4. Growth of Lemna in sterile modified Knop's solution of various phosphate-calcium ratios.

The basal solution used in this experiment was identical with the modified Knop's solution used by Olsen (98) and its composition, along with that of Clark's nutrient solution, is shown in Table VIII.

The modified Knop's solution was prepared from recrystallized salts, and from conductivity water which had been distilled three times; the last distillation was from a Pyrex glass still. To vary the phosphate-calcium ratio as desired the amount of mono-potassium phosphate in each culture was reduced to give the correct quantity of phosphate. In some cases the potassium concentration was brought back to the original amount by adding definite quantities of potassium sulfate or potassium chloride. One hundred cc. of the nutrient solution were prepared from the more concentrated stock solutions, and adjusted to pH's 4.0,

Table VIII. Composition of solutions.

Element or Radical	Modified Knop' solution concentration		Clark's solution concentration	
	Mg./liter	Millimols per liter	Mg./liter	Millimols per liter
Calcium	101.830	2.54	16.034	0.40
Magnesium	19.730	0.81	24.670	1.00
Nitrate	402.480	6.49	496.100	8.00
Sulfate	78.240	0.81	96.420	1.00
Potassium	101.780	2.59	312.800	8.00
Phosphate	104.690	1.10	76.040	0.80
Boron	0.044	0.0040	-----	----
Iron	7.020	0.1260	0.619	0.0100
Manganese	0.123	0.0022	0.025	0.0005
Zinc	0.057	0.0008	----	----
Ammonium	---	---	1.017	0.0590

5.0, 6.0, and 7.0 by adding measured quantities of potassium hydroxide. These solutions were sterilized by autoclaving, allowed to cool for about 24 hours, and the pH was determined by the glass electrode. The cultures which originally had a pH of 4.0 and 5.0 remained at that value for 24 hours, while those at pH's 6.0 and 7.0 became much more acid. To overcome this decrease in pH by autoclaving, excess base was added before sterilizing until a measured quantity of the potassium hydroxide would give a reaction of pH 6.0 and 7.0 or more after the 24 hour period. In all cases a large quantity of precipitate was formed in the solutions at pH's 6.0 and 7.0.

Two series of 40 of these autoclaved solutions, which contained phosphate-calcium ratios varying from Olsen's (98) highest ratio to one fourth of his lowest, were prepared; these ratios are recorded in Table IX along with the concentration of phosphate, calcium, and potassium in each culture.

Non-sterile plants were grown in one series of these sterilized solutions, but as the results were similar to those of the sterile cultures, the data concerning that series are omitted.

Lemna plants, free from micro-organisms, were grown in the solutions of the second series for a period of five weeks; during this time the plants were transferred to freshly

Table IX. Influence of various phosphate-calcium ratios in modified Knop's solution on reproduction and chlorosis in *Lemna*.

Culture No.	Initial pH	Phosphate concentration Millimols per Liter	Calcium concentration Millimols per Liter	Phosphate-Calcium ratio	Potassium concentration Millimols per Liter	KxlOO	Condition of plants
1	4.0	1.10	2.54	0.432	2.59	3.7	normally green
2	5.0	1.10	2.54	0.432	2.59	3.7	" "
3	6.0	1.10	2.54	0.432	2.59	3.5	slight chlorosis in 7 days
4	7.9	1.10	2.54	0.432	2.59	3.5	slight chlorosis in 7 days
5	4.0	0.88	2.54	0.346	2.37	6.7	normally green
6	5.0	0.88	2.54	0.346	2.37	7.2	normally green
7	5.0	0.88	2.54	0.346	2.59	6.2	" "
8	5.0	0.88	2.54	0.346	2.59	5.2	" "
9	6.0	0.88	2.54	0.346	2.37	4.7	slight chlorosis in 7 days
10	6.0	0.88	2.54	0.346	2.59	4.2	" " "
11	6.0	0.88	2.54	0.346	2.59	5.0	" " "
12	7.8	0.88	2.54	0.346	2.37	3.5	" " "
13	4.0	0.66	2.54	0.259	2.12	7.5	normally green

Table IX. (continued).

Culture No.	Initial pH	Phosphate concentration Millimols per Liter	Calcium concentration Millimols per Liter	Phosphate-Calcium ratio	Potassium concentration Millimols per Liter	Kx100	Condition of plants
14	5.0	0.66	2.54	0.259	2.12	6.0	normally green
15	6.0	0.66	2.54	0.259	2.12	4.7	slight chlorosis in 7 days
16	7.6	0.66	2.54	0.259	2.12	3.7	slight chlorosis in 7 days
17	4.0	0.44	2.54	0.173	1.94	7.0	normally green
18	5.0	0.44	2.54	0.173	1.94	6.7	" "
19	6.0	0.44	2.54	0.173	1.94	6.0	slight chlorosis in 7 days
20	7.9	0.44	2.54	0.173	1.94	3.2	slight chlorosis in 7 days
21	4.0	0.33	2.54	0.130	1.82	6.2	normally green
22	5.0	0.33	2.54	0.130	1.82	8.0	" "
23	6.0	0.33	2.54	0.130	1.82	5.5	slight chlorosis in 7 days
24	7.19	0.33	2.54	0.130	1.82	3.0	slight chlorosis in 7 days
25	4.0	0.22	2.54	0.086	1.71	7.0	normally green
26	5.0	0.22	2.54	0.086	1.71	6.8	" "
27	5.0	0.22	2.54	0.086	2.59	6.8	" "

Table IX. (continued).

Culture No.	Initial pH	Phosphate concentration Millimols per Liter	Calcium concentration Millimols per Liter	Phosphate-Calcium ratio	Potassium concentration Millimols per Liter	Kx100	Condition of plants
28	5.0	0.22	2.54	0.086	2.59	6.5	normally green
29	6.0	0.22	2.54	0.086	1.71	4.5	slightly chlorotic in 24 days
30	7.69	0.22	2.54	0.086	2.59	4.0	very chlorotic in 24 days
31	7.70	0.22	2.54	0.086	2.59	4.0	very chlorotic in 24 days
32	7.70	0.22	2.54	0.086	1.71	4.0	very chlorotic in 24 days
33	4.0	0.11	2.54	0.043	1.60	7.2	normally green
34	5.0	0.11	2.54	0.043	1.60	8.5	" "
35	6.0	0.11	2.54	0.043	1.60	5.5	chlorotic in 24 days
36	7.19	0.11	2.54	0.043	1.60	4.5	" " " "
37	4.0	0.055	2.54	0.022	1.54	7.0	normally green
38	5.0	0.055	2.54	0.022	1.54	6.0	" "
39	6.0	0.055	2.54	0.022	1.54	6.0	slight chlorosis in 24 days
40	7.70	0.055	2.54	0.022	1.54	4.5	chlorotic in 24 days

prepared solutions twice weekly at regular 3 and 4 day intervals. Sterile technique was used throughout and the plants were exposed to C.L.T.A. for 14½ hours daily. At each solution renewal the number of fronds was counted and from 10 to 20 fronds were transferred to the new solutions. The general appearance of the plants was observed and recorded at the time of each transfer.

The initial reaction of the cultures, the concentration of the phosphate, calcium, and potassium, and the phosphate-calcium ratio recorded in Table IX along with the rate of reproduction and the general condition of the plants produced at various reactions of the nutrient solution. Cultures 1 to 4 had a phosphate-calcium ratio equal to Olsen's (98) highest ratio and cultures 25-32 furnished a ratio equal to his lowest; cultures 5 to 24 had intermediate ratios, and cultures 33 to 40 had ratios which were smaller than his lowest.

Cultures 1 to 4, representing Olsen's (98) highest phosphate-calcium ratio at pH's 4.0, 5.0, 6.0, and 7.9, gave very poor growth at all reactions; chlorosis developed within seven days in the cultures at pH's 6.0 and 7.9. In cultures 25 to 32, which corresponded to Olsen's low phosphate-calcium ratio, the growth of the plants at pH 4.0 to 5.0 was good during the entire experiment, but the plants at pH's 6.0 and 7.7 became definitely chlorotic in 17 days.

The cultures which contained potassium sulfate, 27 and 30, or potassium chloride, 28 and 31, (added to adjust the potassium concentration to the value in the high phosphate series) showed no marked difference from similar cultures containing a lower concentration of potassium, 26 and 32. The cultures which contained phosphate-calcium ratios between these two series showed the same general trend; the plants developed normally in all cultures at pH's 4.0 and 5.0 and poor growth and chlorosis developed in the more basic reactions. Cultures 33 to 36, which had a phosphate-calcium ratio of one half Olsen's low ratio, gave the same general results, although the chlorosis did not develop until after 24 days in the solutions at pH 6.0 and 7.19. Cultures 37 to 40 which had one fourth the phosphate-calcium ratio of Olsen's low ratio showed the same response as the cultures which contained one half the low ratio.

Since the results of this experiment did not conform with those reported by Olsen (98) for maize, it was decided to check the results by using a solution for the Lemna in which no precipitate was formed, so that the concentration of the elements in contact with the plants would be identical with the amount added to the solution originally.

Experiment 5. Growth of Lemna in modified Clark's solution of various phosphate-calcium ratios.

Clark's nutrient solution, the composition of which is given in Table VIII, was used as the basal stock solution for this investigation. This solution contained 0.4 millimols of calcium and 0.8 millimols of phosphate per liter, with a phosphate-calcium ratio of 2.0. Instead of the usual mono-calcium phosphate, which serves as a source of both calcium and phosphate in this solution, calcium nitrate and mono-potassium phosphate were employed to give these ions. The calcium was used in the concentration of 0.40 millimols per liter, which was shown by Clark (36) to be suitable for the growth of Lemna, and the phosphate was varied from 0.160 millimols to 0.00032 millimols per liter, by varying the quantity of mono-potassium phosphate added to the cultures. Ten cultures were prepared and the pH adjusted to 7.0 by adding experimentally determined quantities of a dilute solution of potassium hydroxide. The final volume was made up to 100 cc. and the solutions were sterilized. No visible precipitate was observed in these solutions after sterilization and standing for 24 hours.

The cultures described were used for growing Lemna free from micro-organisms. These plants were transferred to freshly prepared solutions twice weekly at regular intervals for five weeks. Sterile technique was used throughout and plants were grown in the C.L.T.A. light chamber with 14½ hours illumination daily. The pH's of the media after

growth were determined at the time of each transfer; the average pH of each culture for the duration of the experiment is recorded in Table X.

Graphs of the reproduction rates, K, for the plants grown in solutions of the various phosphate-calcium ratios are shown in Figure 5. The values for K, along with the phosphate-calcium ratios, the concentrations of the phosphate and calcium, and the average pH's of the exhausted solutions are given in Table X.

From the data of Table X it can be seen that the reproduction rate increases as the phosphate-calcium ratio decreases from 0.16 to 0.0130, and then decreases as the ratio became smaller. The plants in cultures one to six inclusive were chlorotic throughout the experiment, with the exception of culture 4 which was chlorotic at first but turned green after the culture became contaminated with micro-organisms. Cultures 7 to 10 which had phosphate-calcium ratios of 0.064 to 0.0008 remained normally green, but the growth was very poor and the roots became very long.

All the plants in the series developed severe chlorosis when the average pH's of the exhausted solutions remained above 5.4, but were normally green when the average pH fell below 5.4. This is particularly noticeable in culture 4 (original pH 7.14) in which there was a contamination after 3 weeks; before the contamination the pH of the cultures

Table X. The influence of various phosphate-calcium ratios in modified Clark's solution on the reproduction and chlorosis in Lemna: new solutions twice weekly.

Culture No.	Original pH	Concentration of phosphate Millimols per liter.	Concentration of calcium Millimols per liter	Phosphate-calcium ratio	Average pH of used solutions at time of transfer	Kx100 from Fig. 5	Condition of plants
1	7.02	0.16000	0.400	0.4000	6.77	3.7	chlorotic throughout experiment
2	7.07	0.08000	0.400	0.2000	6.60	3.9	" "
3	7.14	0.04000	0.400	0.1000	6.55	3.7	" "
4	7.14	0.02000	0.400	0.0500	5.87-5.07	5.2	chlorotic, but became green after a bacterial contamination
5	7.07	0.01000	0.400	0.0250	5.74	5.0	chlorotic throughout experiment
6	7.03	0.00510	0.400	0.0130	5.43	6.2	" "
7	7.07	0.00260	0.400	0.0064	5.00	4.5	normally green, average size, long roots
8	7.07	0.00130	0.400	0.0032	4.71	4.5	normally green, badly bunched, long roots
9	7.05	0.00064	0.400	0.0016	5.13	3.1	" "
10	7.04	0.00032	0.400	0.0008	5.60	2.7	normally green, badly bunched, very long roots

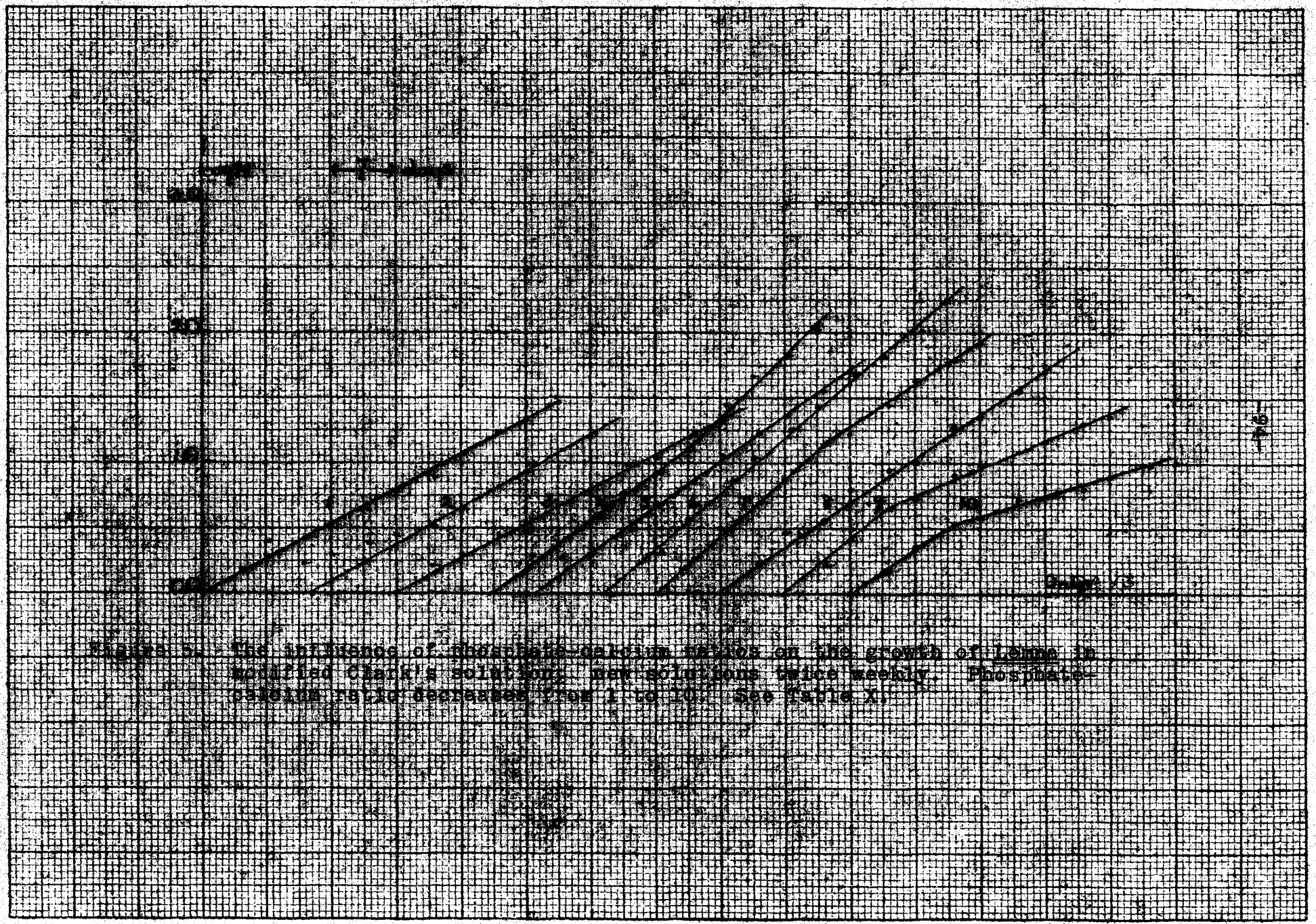

Plate 54. The influence of phosphate-calcium ratios on the growth of Lemna in modified Clark's solution; nine solutions twice weekly. Phosphate-calcium ratio decreases from 1 to 10. See Table XI.

dropped to about 5.87 and the plants were chlorotic, after contamination the pH of the solution dropped to a pH of 5.07 and the plants again developed chlorophyll and showed stimulated growth.

With this observation in mind, a renewal of the series was made, with more frequent changes of the media, to determine whether the low phosphate-calcium ratio or the decrease in pH was the factor which allowed development of normally green plants.

The same solutions and technique were employed as before, except that the plants were transferred to freshly prepared solutions every 24 hours so that the pH of the solutions might be kept more nearly constant. The rates of reproduction, K, were obtained graphically from Figure 6 and are recorded along with the concentrations of the solutions, and the average pH's of the solutions at the end of each days growth in Table XI.

The data in Table XI showed that chlorosis developed in all the cultures regardless of the phosphate-calcium ratio, when the pH's of the solutions were retained at values above 6.0. By renewing the solutions daily the pH's could be kept at values above 6.0 in cultures containing phosphate-calcium ratios from 0.40 to 0.00080.

After using this wide range of phosphate-calcium ratios without any success in preventing chlorosis at pH 7.0, an

Table XI. The influence of various phosphate-calcium ratios in modified Clark's solution on the growth and chlorosis in Lemna: new solutions daily.

Culture No.	Original pH	Concentration of phosphate Millimols per liter	Concentration of calcium Millimols per liter	Phosphate-calcium ratio	Average pH of solutions after 24 hours	Kx100 from Fig. 6	Condition of plants
1	7.02	0.16000	0.400	0.4000	6.85	6.7	chlorotic in 14 days
2	7.07	0.0800	0.400	0.2000	6.75	7.2	" " " "
3	7.14	0.04000	0.400	0.1000	6.77	6.8	" " " "
4	7.14	0.02000	0.400	0.0500	6.57	6.7	" " " "
5	7.07	0.01000	0.400	0.0250	6.53	6.2	" " " "
6	7.03	0.00510	0.400	0.0130	6.48	6.3	" " " "
7	7.07	0.00260	0.400	0.0064	6.43	5.7	" " 21 "
8	7.07	0.00130	0.400	0.0032	6.28	6.1	" " " "
9	7.05	0.00064	0.400	0.0016	6.33	5.4	" " " "
10	7.04	0.00032	0.400	0.0008	6.36	3.7	" " " "

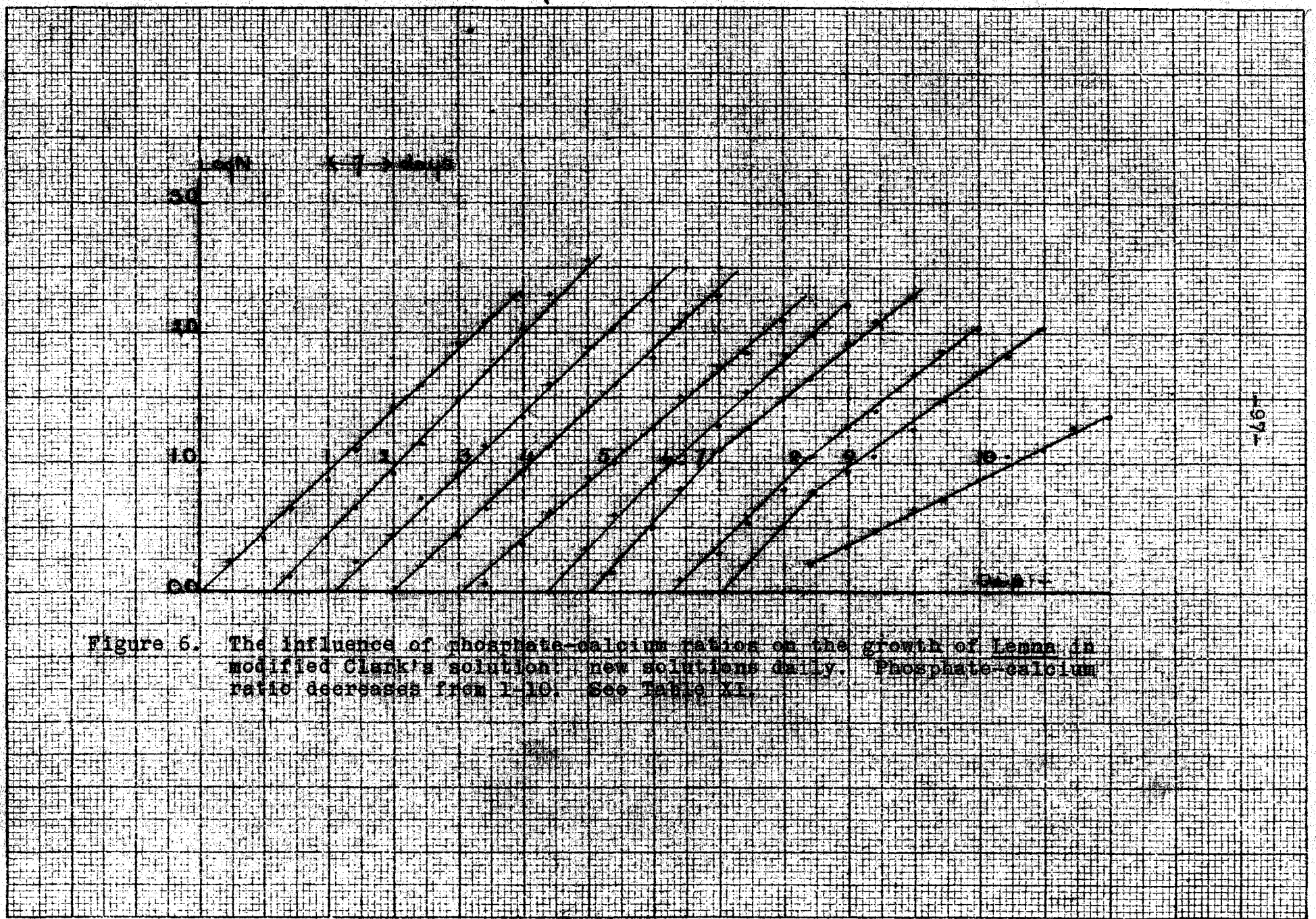

Figure 6. The influence of phosphate-calcium ratios on the growth of Lemna in modified Clark's solution. new solutions daily. Phosphate-calcium ratio decreases from 1-10. See Table XI.

attempt was made to duplicate Olsen's (98) findings in non-sterile solutions.

Experiment 6. The effectiveness of various phosphate-calcium ratios in the prevention of chlorosis in Lemna at different reactions in non-sterile cultures.

The modified Knop's solution as given in Table VIII, and a similar solution containing one fifth the quantity of phosphate, were used in this experiment; the phosphate-calcium ratios were 0.432 and .086 respectively. Clark's solution was also used and was altered, as in Experiment 5, to give two series of 0.400 and 0.080. The media were prepared from stock solutions and were adjusted to pH's 4.8, 6.0, 7.0, and 8.0 by adding measured quantities of potassium hydroxide.

Eight to ten normally green Lemna fronds were placed in 100 cc. of each of these solutions and exposed to mazda illumination at 25° C. for 14½ hours daily. The plants were transferred to freshly prepared solutions each day for one week, at which time the number of plants in each culture was reduced to 16 fronds and the daily transfers to new solutions continued for an additional 10 days. At the end of the 17 days the plants of each culture were removed to petri dishes and a photograph was made of the entire series to show the state of the plants.

The condition of the plants along with the concentration of the calcium and phosphorus, the phosphate-calcium ratios, the initial reactions of the solutions and the rate of reproduction, K, are recorded in Table XII.

The data in Table XII show that all cultures with an initial pH of 4.8 developed normally for a period of seventeen days (Plate I). All cultures which had initial pH's of 7.0 and 8.0 developed severe chlorosis within seven days, although the reproduction rate in modified Clark's solution was in most cases about equal to that of the more acid reaction. In the Knop's solution the plants in the culture at pH 6.0 were only slightly chlorotic at the end of 17 days, while those in the culture with the ratio of 0.086 were normally green; also, the pH of the solution with the high ratio fell to a value of 5.90, while the pH with the low ratio fell to 5.55. In the modified Clark's solution at pH 6.0 the plants were normally green at both ratios.

This attempt to duplicate Olsen's (98) results with Lemna in non-sterile media was unsuccessful; the plants in both Knop's and Clark's solutions of different phosphate-calcium ratios became chlorotic and growth was inhibited when the initial reactions were more alkaline than pH 6.0. This variation in the results can hardly be attributed to the differences in the technique used. Olsen used 500 cc. cultures in which the reactions were adjusted daily with

Table XII. Growth of Lemna in non-sterile cultures of various phosphate-calcium ratios: daily change.

Culture No.	Solution name	Original pH	Average pH of solutions at end of 24 hours	Calcium concentration Millimols per liter	Phosphate concentration Millimols per liter	Phosphate-calcium ratio	Kx100	Appearance of plants (see Plate 1)
1	Knop	4.8	4.62	2.54	1.100	0.432	7.1	normally green, average size
2	"	6.0	5.91	2.54	1.100	0.432	5.2	slight chlorosis in 17 days
3	"	7.0	6.96	2.54	1.100	0.432	3.5	definitely chlorotic in 7 days
4	"	8.0	7.23	2.54	1.100	0.432	3.2	" " "
5	"	4.8	4.50	2.54	0.220	0.086	7.5	normally green, average size
6	"	6.0	5.55	2.54	0.220	0.086	7.0	" "
7	"	7.0	6.84	2.54	0.220	0.086	6.0	definitely chlorotic in 7 days
8	"	8.0	7.31	2.54	0.220	0.086	5.0	" " "

Table XII. (continued).

Culture No.	Solution name	Original pH	Average pH of solutions at end of 24 hours	Calcium concentration Millimols per liter	Phosphate concentration Millimols per liter	Phosphate-calcium ratio	Kx100	Appearance of plants (see Plate 1)
9	Clark	4.8	4.55	0.40	0.160	0.400	5.0	normally green, average size
10	"	6.0	5.67	0.40	0.160	0.400	5.5	definitely chlorotic in 7 days
11	"	7.0	6.52	0.40	0.160	0.400	5.5	" " "
12	"	8.0	7.07	0.40	0.160	0.400	4.5	" " "
13	"	4.8	4.67	0.40	0.032	0.080	6.3	normally green, average size
14	"	6.0	4.93	0.40	0.032	0.080	5.8	" "
15	"	7.0	6.65	0.40	0.032	0.080	5.5	very chlorotic in 7 days
16	"	8.0	6.65	0.40	0.032	0.080	5.7	" "

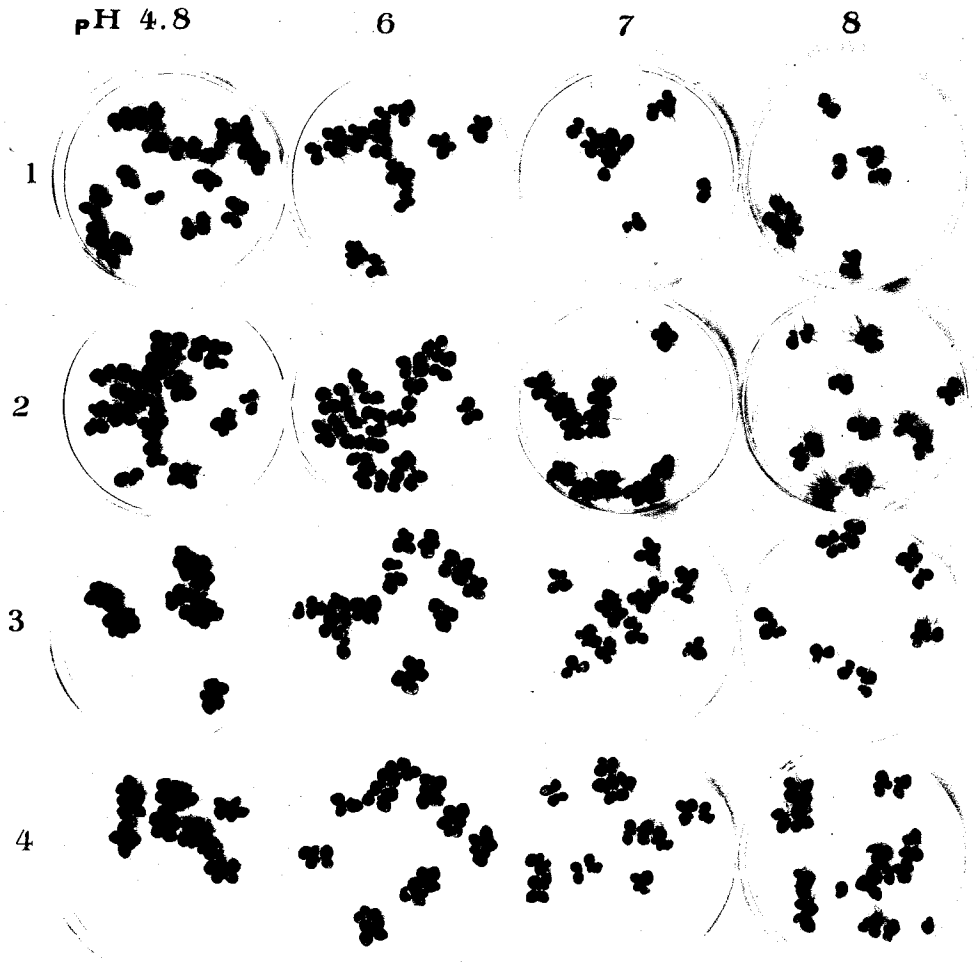

Plate I. Growth of Lemna in non-sterile nutrient solutions with various phosphate-calcium ratios.
(Horizontal row 1, cultures 1-4 of Table XII.)
(Horizontal row 2, cultures 5-8 of Table XII.)
(Horizontal row 3, cultures 9-12 of Table XII.)
(Horizontal row 4, cultures 13-16 of Table XII.)

renewals every five days, while in this investigation the culture volume was 100 cc. with daily renewals. Light and temperature may be the clue to the difference, but Olsen did not record these conditions. Under the conditions used in the experiments reported here Lemna did not reproduce in alkaline media without chlorosis appearing unless organic matter was present.

DISCUSSION

During the early part of the present century several investigators believed that there existed organic compounds which were essential for the normal development of plants, in the same way that vitamins were considered essential for the normal development of animals; to these unidentified organic substances Bottomley (12-210) assigned the name "auximone", which means growth-promoting. He suggested that these auximones were present in the seeds of plants, in the organic matter of the soil, and in treated peat. Both Bottomley and Mockeridge (93, 94) found that Lemna failed to grow and reproduce in strictly inorganic solutions, but that growth was satisfactory when organic materials such as manure, peat, or soil were added to these solutions.

The conclusions, which Bottomley drew from his experiments with Lemna and other plants, were not confirmed by other investigators. Clark (34, 36), Clark and Roller (39, 40), Wolfe (137), Saegar (110), and Ashby (7) found that Lemna would grow and reproduce normally in nutrient solutions devoid of organic matter when the solutions were properly balanced. In general, these workers reported that small quantities of organic matter tended to stimulate plant growth, but were not essential; Clark showed that sometimes

organic matter did not affect the Lemna, and that occasionally the growth was depressed.

Other investigators have successfully grown seedlings in strictly inorganic media of various compositions without any noticeable adverse influence on the growth, but their success might be challenged since, in most cases, the experiments were carried out under non-sterile conditions and in the presence of the seed from which the plant was produced; both bacteria and the seed were considered by Bottomley (12, 20) to be a source of "auximones". For this investigation Lemna major was used as the test plant because it could be grown under controlled conditions in sterile nutrient solution (38), and it had no seed from which to receive stimulation.

Olsen (97, 98) observed that the reproduction of Lemna was stimulated by alkaline humus extracts at reactions more basic than pH 6.0; he attributed this stimulation to the presence of a complex organic iron molecule from which the iron became available to plants at reactions at which inorganic iron was known to be assimilated with difficulty. Olsen carried on his investigations under non-sterile conditions; it is at least possible that his results may have been influenced by the presence of both bacteria and organic matter and not by the organic matter alone. Burk et al. (28) reported that the stimulation of growth of

azotobacter and tomatoes was also proportional to the iron content of the humic acids added to the nutrient solutions.

In this investigation an alkaline humus extract, (iron humate), containing a known quantity of iron, was used as a part of the sterile nutrient solutions in which the Lemna, free from micro-organisms, were grown. The amounts of the iron humate, and the total iron-iron humate ratios, were varied in order to find the effect of both iron and organic matter on the growth. Since Olsen (97, 98) observed that the stimulative effect of alkaline peat extracts was most manifest at pH 7.0, the plants were grown at various reactions from pH 3.5 to 9.0.

In all cases, the presence of iron humate in cultures more acid than pH 5.0 caused a depression of the growth of Lemna, regardless of the concentration of the iron or the iron humate; however, at pH 6.0 and above, the growth was stimulated greatly by additions of iron humate. That a definite iron-organic matter balance is needed for maximum stimulation of plant growth was shown by the fact that stimulation was decreased when either the iron concentration was decreased, or when the organic matter was increased above certain limits; the maximum growth was obtained in the culture having a total iron-iron humate ratio of 0.002, and a total concentration of iron of 0.62 mg. per liter at pH 7.0. In solutions at 7.0 the iron humate was completely

effective in the prevention of chlorosis; as the reaction became more alkaline (pH 8.0-9.0) slight chlorosis developed, but this was not comparable to the chlorosis in cultures containing no organic matter.

Whether the iron is present as a complex molecule which can be assimilated directly, as postulated by Olsen (98), or as a complex molecule which ionizes to give available iron-ions, as Hopkins (70) believed, is a subject for further experimentation. In contrast to this, the theory, also suggested by Olsen (98), that the iron is present in iron humate in a manner similar to the iron in ferric citrate, can be questioned, since it was observed that the citrate, with the same concentration of iron, was not effective in preventing chlorosis at pH's above 6.0 but actually gave optimum growth response at pH 5.0. This conformed with the findings of Fly (49) who observed that the optimum pH for the growth of Lemna depended upon the quantity of ferric citrate present. Fly found that the optimum pH progressed from pH 5.0 with 0.5 mg. of Fe per liter to 7.8 with 32 mg. of Fe per liter.

There was some indication that the iron humate may have acted by furnishing available iron ions as postulated by Hopkins. It was observed that the total iron-iron humate ratio was a factor in the effectiveness of the organic matter in stimulating growth at pH 7.0; the optimum growth was

obtained at a ratio of 0.002 for a total concentration of iron of 0.62 mg. per liter. If the iron was added in excess, causing a decrease in this ratio, the growth was inhibited at pH 7.0 and the optimum reaction for growth was moved from pH 7.0 to 6.0, thereby indicating that the excess of iron may have decreased the ionization of the iron humate molecule, and this in turn decreased the availability of the iron. An increase of the organic matter with the iron remaining constant also caused a depression of growth at pH 7.0 and this could be explained by the same reasoning. This theory seemed to have more support in the experimental results reported here than the theory that complex organic iron-humate molecules were absorbed directly by the plant, since an increase either of iron or of organic matter depressed the growth. A third possibility may be suggested. The organic matter may serve as a colloid which adsorbs the iron and releases it to the plant as the inorganic iron salt, but to prove any of the theories would need far more data than were obtained.

The influence of organic matter on plant growth depends upon the reaction of the solution to which it has been added and upon the type of organic matter. In the nutrient solution used in this experiment it was clear that the iron humate was essential for the normal development of Lemna at reactions above pH 6.0 because of its property to make iron available

to the plant, and that it was unessential and even undesirable at reactions in which the inorganic iron is available--pH 4.5 to 5.0.

Olsen (98) reported that proper adjustment of the physiological balance--the phosphate-calcium ratio--precluded the need for organic matter to prevent chlorosis in plants grown at neutral reactions. By the adjustment of the phosphate-calcium ratio to a value of 0.086 in terms of millimols per liter, he was able to grow maize in nutrient solutions containing no added organic matter without any evidence of chlorosis at pH's 6.0 or 7.0. He attributed the appearance of chlorosis in plants grown in nutrient solutions of high phosphate-calcium ratios to the precipitation of the iron in the vascular bundles of the plant as ferric phosphate, and not to the unavailability of the iron in alkaline solutions. Theron (125), on the other hand, determined the pH of the expressed root juices of several plants and concluded that the concentration of the hydroxyl ion was hardly sufficient to prevent translocation of the absorbed iron.

In this investigation several attempts were made to establish the optimum phosphate-calcium ratio for Lemna at neutral reactions with the hope of eliminating the necessity for keeping the reactions of the inorganic solutions at pH 4.5 to 5.0. If this could be accomplished, the influence of organic matter on growth, apart from its influence in making

iron more available for plant assimilation in neutral cultures, could be further investigated.

In Experiment 4, Knop's solution with phosphate-calcium ratios varying from 0.432 to 0.022 was used for Lemna cultures at pH's 4.0, 5.0, 6.0, and 7.0-8.0. The sterile plants were grown in these cultures for five weeks, and it was observed that in every case the plants became chlorotic when the initial reactions of the solutions were pH 6.0 or above; however, the chlorosis was delayed in its appearance in the cultures having the lower ratios. The solutions which were adjusted to initial reactions of pH's 6.0 and 7.0 before sterilization became much more acid after sterilization. To overcome this decrease in pH, enough potassium hydroxide was added to give the desired reaction after sterilization, but in a number of cases the pH's of the solutions approached a value of 10 before sterilization. It was thought that this procedure might have caused almost complete precipitation of the iron, and this in turn caused the appearance of the chlorosis, although Olsen (98) observed that as great a quantity of iron was in solution at pH 8.0 and 9.0 as at pH 6.0 and that Lemna developed normally at pH 8.0 and 9.0.

To eliminate this undesirable feature which was required for the Knop's solution, and to develop a nutrient solution in which no precipitate was formed at pH 7.0, Clark's solution was modified to give various phosphate-calcium ratios from

0.400 to 0.0008 (expressed in millimols). The reaction of these solutions was adjusted to pH 7.0 and autoclaved; it was observed that there was no drop in the pH or formation of a visible precipitate after sterilization.

Sterile Lemna grown in these solutions became chlorotic in every case when the pH of the solutions remained above 6.0, but the plants developed into normally green plants in the solutions in which the pH dropped below 5.7 before renewal. Since these solutions were renewed only twice weekly more frequent renewals were desired in order to prevent the increase in acidity in the solution containing the lower quantities of phosphate. The solutions were therefore renewed daily with the result that the plants became chlorotic in the entire series, regardless of the phosphate-calcium ratio; the reaction of the solutions did not drop below pH 6.0 at any time. The rate of reproduction became less when the phosphate concentration was reduced and this was due apparently to the lack of available phosphorus.

Non-sterile Lemna were grown in both Knop's and Clark's solutions with phosphate-calcium ratios of 0.4 and 0.08 at pH's 4.8, 6.0, 7.0, and 8.0 with daily renewal of the solutions. In this case, as in all others, the plants became chlorotic in solutions in which the pH was maintained above pH 6.0. These results do not confirm the findings of Olsen (98) who reported successful growth of Lemna in solutions with an

initial pH of 8.0 without appearance of chlorosis.

The major difference between the technique used in this investigation and that employed by Olsen was the solution's volume and renewal; he grew the plants in 500 cc. of solution which was adjusted daily to the desired pH and renewed every five days, while in this investigation the volume of the solution was 100 cc. and renewed daily. This difference in procedure should hardly have warranted the marked divergence in results. Light conditions however, were not the same, and the condition of light and temperature to which the plants are exposed is known to have an effect upon the appearance of chlorosis. Gericke (53) reported that high intensity of light caused more marked chlorosis, and Sideris and Krauss (118) observed that high temperature increased its severity. The light intensity here was low--200 foot candles--and the temperature constant at 25° C. Olsen's observation that maize grew well for 18 days in solutions with low phosphate-calcium ratios at pH 6.0-7.0 might be attributed to (a) the presence of the necessary iron in the remaining portion of the seed, (b) to bacterial decomposition of this seed to form organic compounds which made the inorganic iron in the solution available for plant assimilation, or (c) to the low iron requirement of the plant; no satisfactory explanation of the difference with the Lemna can be offered.

The use of ammonium nitrogen in place of the more common

nitrate nitrogen in nutrient solutions was shown by Jones (76) to be effective in preventing chlorosis of green plants in neutral or alkaline reactions. This procedure was not attempted in this investigation and it would need to be studied systematically under sterile conditions and controlled environment before it could be stated that it would be effective for the prevention of chlorosis in Lemna.

In all the investigations reported here the conditions of growth have been controlled within certain limits in order to allow interpretation of the variable factors. Temperature, light intensity, light quality, duration of light exposure, and humidity have been controlled, and all but one of the experiments were conducted under sterile conditions to preclude the effect that micro-organisms might have on growth.

In a preliminary part of this investigation a method for the determination of iron was reported. This method combined the wet oxidation of organic matter with the colorimetric determination of the iron by the use of 7-iodo-8 hydroxy-quinoline-5-sulfonic acid as an indicator for the ferric iron. This method has a marked advantage over other colorimetric methods for iron in that the color produced by the dye is constant for at least 25 days and would thereby be applicable to routine water analysis or similar investigations. Another advantage of this method is the high sensitivity; it is not difficult to distinguish between samples differing

by 0.002 mg. of iron in 100 cc. of the solution--2 parts in 100,000,000--and this sensitivity could probably be increased by using a photo-electric cell colorimeter.

The influence of light quality and intensity was studied as a secondary part of this investigation. For the same intensity of light it seemed that illumination with combined red and blue neon light showed a marked advantage over illumination with red alone. A point that must be considered in this connection is the fact that the instrument used to measure the light intensities was standardized against the light produced by a tungsten filament at 3,000° C. It is therefore somewhat doubtful if the results obtained by the use of this instrument were very reliable when applied to the red or blue neon lights; hence, the interpretation of the data must be made with limitations. That this instrument would give relative results when exposed to the same light source is probable, and the data obtained at various intensities from the same source can be compared with some degree of accuracy.

In the experiment in which cultures of sterile Lemna were exposed to various intensities of red and blue neon light it was shown that the rate of growth increased progressively as the intensity of the light increased. This observation conforms with the results reported by other investigators (105)(115).

SUMMARY

Alkaline humus extract--the iron humate--was prepared by extracting a peat soil with a potassium hydroxide solution. This iron humate was "purified" by precipitation with hydrochloric acid and re-dissolving the resulting precipitate in potassium hydroxide.

The iron content of the iron humate was determined by a technique which involved two procedures; (a) the oxidation of the organic matter with hydrogen peroxide in acid solution, and (b) determination of the iron colorimetrically by the use of 7-iodo-8-hydroxyquinoline-5-sulfonic acid as an indicator.

Lemna free from micro-organisms was grown in sterile cultures containing various quantities of iron humate and iron and having a wide range of reactions. Growth was stimulated and chlorosis prevented at reactions more alkaline that pH 6.0, and growth was depressed at pH's 4.5 and 5.0.

Under sterile conditions Lemna was exposed to various intensities and qualities of light produced by neon lights. The rate of reproduction was found to increase as the intensity increased; combined red and blue illumination was more effective than red alone at the same intensity.

None of the phosphate-calcium ratios tried was able to prevent chlorosis in either sterile or non-sterile cultures of Lemna major when the pH's of the solutions were maintained at a value of 6.0 or above.

CONCLUSIONS

1. Iron humate was effective in the prevention of chlorosis of Lemna in neutral reactions but not completely in alkaline reactions.

2. The effectiveness of iron humate in promoting growth and preventing chlorosis of Lemna at neutral reactions was attributed to its power to make iron available for assimilation by the plant.

3. Maximum stimulation of growth was obtained when the iron-organic matter ratios were within the limits 0.001 and 0.005 and the concentration of the iron was 0.01 millimols per liter.

4. Iron humate depressed the growth of Lemna at pH values of 4.5 to 5.0.

5. The growth of Lemna was prohibited by solutions with pH values of 3.5 to 4.0.

6. Cultural solutions containing ferric chloride caused chlorosis of Lemna at reactions more alkaline that pH 6.0, irrespective of the phosphate-calcium ratio.

7. Ferric citrate, at a concentration of 0.62 mg. of iron per liter, would not prevent chlorosis with reactions more alkaline than pH 6.0.

8. Lemna will grow satisfactorily under neon light and neon-mercury light. The rate of reproduction varied with the intensity. There is some indication that the neon illumination is more effective than the light from mazda lamps for the same foot candle power.

9. The 7-iodo-8-hydroxyquinoline-5-sulfonic acid method for the determination of iron is more satisfactory than other colorimetric methods for iron. Combined with the wet oxidation method by hydrogen peroxide, it can be used to measure very small quantities of iron in organic compounds or mixtures.

LITERATURE CITED

(1) Adams, J. E. Chemical investigations of the effect of fertilizer ratios and green manures on yields and composition of crops and the organic matter in Norfolk sand. Ph.D. Thesis, Ia. State Col. 1936.

(2) Allison, R. V. The relation of aeration to development of the soybean plant in artificial culture. N. J. Agr. Exp. Sta. Ann. Rpt. 1921:338-344. 1921.

(3) Andrews, F. M. The effect of aeration on plants. Proc. Ind. Acad. Sci. 1920:147-148. 1920.

(4) Andrews, F. M., and Beals, C. C. The effect of soaking in water and of aeration on the growth of Zea mays. Bull. Torrey Bot. Club 46:91-100. 1919.

(5) Arndt, C. H. The growth of field corn as affected by iron and aluminum salts. Amer. Jour. Bot. 9:47-71. 1922.

(6) Ashby, E. The interaction of factors in the growth of Lemna. III. The interrelationship of duration and intensity of light. Ann. Bot. 43:331-354. 1929.

(7) Ashby, E. The interaction of factors in the growth of Lemna. IV. The influence of minute quantities of organic matter upon growth and reproduction. Ann. Bot. 43:805-816. 1929.

(8) Ayres, A. H. Influence of the composition and concentration of the nutrient solution on plants grown in sand cultures. Univ. Calif. Pub. Agr. Sci. 1:341-394. 1917.

(9) Barnette, R.M. The influence of volume upon the rate of change in the hydrogen-ion concentration of nutrient solutions in contact with plant roots and the effect of this change upon iron availability. N. J. Agr. Exp. Sta. Ann. Rpt. 1921:345-347. 1921.

(10) Barnette, R. M., and Shive, J. W. The influence of solution volume upon plant growth in relation to reaction change and iron availability in culture solutions. Soil Sci. 15:413-425. 1923.

(11) Beal, C. C. The effect of aeration on the roots of Zea mays. Proc. Ind. Acad. Sci. 1917:177-180. 1917.

(12) Blackman, V. H. Plants in relation to light and temperature. I. Effects of light. Jour. Roy. Hort. Soc. 59:1-13. 1934.

(13) Bottomley, W. B. Some effects of humates on plant growth. Rpt. Brit. Assn. Adv. Sci. 1912:680. 1912.

(14) _____. Ammonium humate as a source of nitrogen for plants. Rpt. Brit. Assn. Adv. Sci. 1913:706-707. 1913.

(15) _____. The effect of soluble humates on nitrogen fixation and plant growth. Rpt. Brit. Assn. Adv. Sci. 1913:777-778. 1913.

(16) _____. The significance of certain food substances for growth. Ann. Bot. 28:531-540. 1914.

(17) _____. Some accessory factors in plant growth and nutrition. Proc. Roy. Soc. London, B, 88:236-257. 1914.

(18) _____. A bacterial test for plant food accessories (Auximones). Proc. Roy. Soc. London, B, 88:102-108. 1915.

(19) _____. Some effects of organic growth-promoting substances (Auximones) on the growth of Lemna minor in mineral culture solutions. Proc. Roy. Soc. London, B, 89:481-507. 1917.

(20) _____. The growth of Lemna plants in mineral solutions and in their natural medium. Ann. Bot. 34: 345-352. 1920.

(21) _____. The effect of organic matter on the growth of various water plants in culture solution. Ann. Bot. 34:353-365. 1920.

(22) Breazeale, J. F. Effect of the concentration of the nutrient upon wheat cultures. Science, n. s. 22: 146-149. 1905.

(23) Brenchley, Winifred E. The effect of the concentration of the nutrient solution on the growth of barley and wheat in water cultures. Ann. Bot. 30:77-90. 1916.

(24) Brown, D. D. The effect of different proportions of calcium nitrate and potassium di-hydrogen. Soil Sci. 26:441-446. 1928.

(25) Bryan, O. C. The effect of different reactions on the growth and nodule formation of soy beans. Soil Sci. 13:272-302. 1922.

(26) Bryan, O. C. The effect of different reactions on the growth and calcium content of oats and wheat. Soil Sci. 13:375-382. 1922.

(27) _____. Effect of reaction on growth, nodule formation, and calcium content of alfalfa, alsike clover, and red clover. Soil Sci. 15:23-27. 1923.

(28) Burk, D., Lineweaver, H., and Horner, C. K. Iron in relation to the stimulation of growth by humic acid. Soil Sci. 33:413-451. 1932.

(29) Burkholder, P. R. The rôle of light in the life of plants. Bot. Rev. 2:1-52, 97-172. 1936.

(30) Chapman, G. W. The relation of iron and manganese to chlorosis in plants. New Phytol. 30:266-283. 1931.

(31) Clark, H. E., and Shive, J. W. Influence of continuous aeration upon growth of tomato plants in solution cultures. Soil Sci. 34:37-41. 1932.

(32) _____, and _____. The influence of the pH of a culture solution on the rates of absorption of ammonium and nitrate nitrogen by the tomato plant. Soil Sci. 37:203-225. 1934.

(33) _____, and _____. Influence of the pH of a culture solution on the assimilation of ammonium and nitrate nitrogen by the tomato plant. Soil Sci. 37:459-476. 1934.

(34) Clark, N. A. The soil organic matter and growth-promoting accessory substances. Jour. Ind. Eng. Chem. 16:249-250. 1924.

(35) _____. The rate of reproduction of Lemna major as a function of intensity and duration of light. Jour. Phys. Chem. 29:935-941. 1925.

(36) _____. Plant growth-promoting substances, hydrogen ion concentration and the reproduction of Lemna.

Plant Physiol. 1:273-279. 1926.

(37) _____. Purification of chemicals for use in the preparation of nutrient solutions. I. The crystallization of phosphoric acid. Proc. First Int. Cong. Soil Sci. 3:300-304. 1927.

(38) _____. Technique for the growth of Lemna under sterile conditions with controlled temperature and light. Ia. State Col. Jour. Sci. 7:13-16. 1932.

(39) _____, and Roller, E. M. Auximones and the growth of the green plant. Soil Sci. 17:193-198. 1924.

(40) _____, and _____. The stimulation of Lemna major by organic matter under sterile and non-sterile conditions. Soil Sci. 31:299-308. 1931.

(41) Corson, G. E., and Bakke, A. L. The use of iron in nutrient solution for plants. Proc. Iowa Acad. Sci. 24:477-482. 1917.

(42) Crist, J. W. Growth of lettuce as influenced by reaction of culture medium. Mich. Agr. Exp. Sta. Tech Bul. 71:1-25. 1925.

(43) Davidson, O. W., and Shive, J. W. Influence of the hydrogen ion concentration of the culture solution upon the absorption and assimilation of nitrate and ammonium nitrogen by peach trees grown in sand cultures. Soil Sci. 37:357-385. 1934.

(44) Davis, A. R., and Hoagland, D. R. Further experiments on the growth of plants in a controlled environment. I. The relation of light intensity and exposure time to yield. II. The interrelationship of temperature and light. Amer. Jour. Bot. 15:624-651. 1928.

(45) Deuber, C. G. Potassium ferrocyanide and ferric ferrocyanide as sources of iron for plants. Soil Sci. 21: 23-26. 1925.

(46) Duggar, B. M. Hydrogen ion concentration and the composition of nutrient solutions in relation to growth of seed plants. Ann. Mo. Bot. Gard. 7:1-50. 1920.

(47) Eisenmenger, W. S. Toxicity, additive effects, and

Antagonism of salt solutions as indicated by growth of wheat roots. Bull. Torrey Bot. Club 55:261-304. 1928.

(48) Eltinge, Ethel T. The effect of ultraviolet radiation upon higher plants. Ann. Mo. Bot. Gard. 15:169-240. 1928.

(49) Fly, C. L. Organic iron and hydrogen-ion concentration as associated factors affecting the rate of reproduction of Lemna major. Proc. Okla. Acad. Sci. 15:77-80. 1935.

(50) Garner, W. W., and Allard, H. A. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. 18:553-606. 1920.

(51) Gericke, W. F. Influence of temperature on the relations between nutrient salt proportions and the early growth of wheat. Amer. Jour. Bot. 8:59-62. 1921.

(52) _____. On the physiological balance in nutrient solutions for plant cultures. Amer. Jour. Bot. 9: 180-182. 1922.

(53) _____. Effect of light on availability of iron to wheat plants in water cultures. Bot. Gaz. 79:106-108. 1925.

(54) Gile, P. L. Relation of calcareous soils to pineapple chlorosis. Porto Rico Agr. Expt. Sta. Bull. 11:5-45. 1911.

(55) _____. Lime-magnesia ratio as influenced by concentration. Porto Rico Agr. Expt. Sta. Bull. 12:1-24. 1913.

(56) _____, and Carrero, J. O. Assimilation of colloidal iron by rice. Jour. Agr. Res. 3:205-210. 1914.

(57) _____, and _____. Assimilation of iron by rice from certain nutrient solutions. Jour. Agr. Res. 7:503-528. 1916.

(58) _____, and _____. Cause of lime-induced chlorosis and availability of iron in the soil. Jour. Agr. Res. 20:33-62. 1920.

(59) Gris, E. Nouvelles expériences sur l'action des composés ferrugineux solubles, appliqués á la végetation et spécialment au traitement de la chlorose et de la débilité des plantes. Comp. Rend. Acad. Sci. (Paris). 19:18-19. 1844.

(60) Hahn, P. F., and Whipple, G. H. II. Iron metabolism. Its absorption, storage, and utilization in experimental anemia. Amer. J. Med. Sci. 191:24-42. 1936.

(61) Hall, A. D., Brenchley, W. E., and Underwood, L. M. The soil solution and the mineral constituents of the soil. Phil. Trans. Roy. Soc. B. 204:179-200. 1914. Reprinted in Jour. Agr. Sci. 6:278-301. 1914.

(62) Hanna, W. F. Growth of corn and sunflowers in relation to climatic conditions. Bot. Gaz. 78:200-214. 1924.

(63) _____. The nature of growth rate in plants. Sci. Agr. 5:133-138. 1925.

(64) Hartwell, B. L., and Pember, F. R. The relative toxicity of ferrous sulfate to barley and rye seedlings. R. I. Agr. Expt. Sta. 21st Ann. Rept. 1908: 286-284. 1908.

(65) Hixon, R. M. The effect of the reaction of a nutritive solution on germination and the first stages of plant growth. Medd. Vetenskapsak. Nobel institut 4:1-28. 1920.

(66) Hoagland, D. R. The effect of hydrogen and hydroxyl ion concentration on the growth of barley seedlings. Soil Sci. 3:547-560. 1917.

(67) _____. The relation of the plant to the reaction of the nutrient solution. Science, n.s. 48:422-425. 1918.

(68) _____. Relation of concentration and reaction of the nutrient medium to the growth and absorption of the plant. Jour. Agr. Res. 18:73-117. 1919.

(69) _____. The effect of the plant on the reaction of the culture solution. Calif. Agr. Exp. Sta. Tech. Paper. 12:1-16. 1923.

(70) Hopkins, E. F. Iron-ion concentrations in relation to growth and other biological processes. Bot. Gaz. 89:209-240. 1930.

(71) _____, and Wann, F. B. Relation of hydrogen-ion concentration to growth of Chlorella and to the availability of iron. Bot. Gaz. 81:353-376. 1926.

(72) _____, and _____. Iron requirement for Chlorella. Bot. Gaz. 84:407-427. 1924.

(73) Ingalls, R. H., and Shive, J. W. Relation of H-ion concentration of tissue fluids to distribution of iron in plants. Plant Physiol. 6:103-125. 1931.

(74) Janssen, G. The comparative acid tolerance of some southern legumes. Soil Sci. 27:469-498. 1929.

(75) Johnson, E. S. Comparative study of the six types of nutrient solutions in relation to the growth of potato plants in sand cultures. Soil Sci. 20:347-401. 1925.

(76) Jones, L. H. The effect of ammonium sulfate upon the availability of iron in nutrient solutions. N. J. Agr. Exp. Sta. Ann. Rpt. 1920:405-409. 1920.

(77) _____, and Shive, J. W. The influence of iron in the forms of ferric phosphate and ferrous sulfate upon the growth of wheat in nutrient solution. Soil Sci. 11:93-99. 1921.

(78) _____, and _____. Effect of ammonium sulfate upon plants in nutrient solutions supplied with ferric phosphate and ferrous sulfate as sources of iron. Jour. Agr. Res. 21:701-728. 1921.

(79) _____, and _____. Influence of wheat seedlings upon the hydrogen ion concentration of nutrient solutions. Bot. Gaz. 73:391-400. 1922.

(80) _____, and _____. Influence of ammonium sulfate on plant growth in nutrient solutions and its effect on hydrogen ion concentration and iron availability. Ann. Bot. 37:353-377. 1923.

(81) Kempton, J. H. The rate of growth of green and albino maize seedlings. Jour. Agr. Res. 29:311-312. 1924.

(82) Koch, F. C., and McMeeken, T. L. A new direct nesslerization micro-Kjeldahl method and a modification of the Nessler-Folin reagent for ammonia. J. Am. Chem. Soc. 46:2066-2069. 1924.

(83) Leitch, I. Some experiments on the influence of temperature on the rate of growth in Pisum sativum. Ann. Bot. 30:25-46. 1916.

(84) Lehenbauer, P. A. Growth of maize seedlings in relation to temperature. Physiol. Res. 1:247-288. 1914.

(85) Livingston, B. E. Further studies on the properties of unproductive soils. U. S. Dept. Agr. Bur. Soils Bul. 36. 1907.

(86) _____. Notes and comment on Gericke's work on temperatures and germination. Plant World 22: 26-27. 1919.

(87) _____, and Tottingham, W. E. A new three-salt nutrient solution for plant cultures. Amer. Jour. Bot. 5:337-347. 1918.

(88) Loo, T. L. The effect of renewal of nutrient solutions upon the growth of culture plants and its relation to aeration. Japan Jour. Bot. 4:71-96. 1929.

(89) McCall, A. G., and Haag, J. R. The relation of hydrogen-ion concentration of nutrient solutions to growth and chlorosis of wheat plants. Soil Sci. 12:69-77. 1921.

(90) Mac Dougal, D. T. Growth of wheat (Triticum) and corn (Zea). Carnegie Inst. Wash. Year Book 16:83-87. 1917.

(91) Marsh, R. P., and Shive, J. W. Adjustment of iron supply to requirements of soy beans in solution culture. Bot. Gaz. 79:1-27. 1925.

(92) Meier, H. F. A., and Halstead, C. E. Hydrogen-ion concentration relations in a three-salt solution. Soil Sci. 11:325-352. 1921.

(93) Mockeridge, Florence A. The occurrence and nature of plant growth-promoting substances in various organic manurial composts. Biochem. Jour. 14:432-450. 1920.

(94) _____. The formation of plant growth-promoting substances by micro-organisms. Ann. Bot. 38:723-734, 1924.

(95) Morris, L. S., and Crist, J. W. The influence of reaction of culture medium on growth of strawberry plants. Mich. Agr. Exp. Sta. Tech. Bul. 77:1-16. 1927.

(96) Newton, J. D. The selective absorption of inorganic elements by various crop plants. Soil Sci. 26:85-91. 1928.

(97) Olsen, C. On the influence of humus substances on the growth of green plants in water culture. Compt. Rend. Lab. Carlsberg. 15:1-16. 1929.

(98) _____. Iron absorption and chlorosis in green plants. Compt. Rend. Lab. Carlsberg. 21:1-52. 1935.

(99) Osterhout, W. J. V. On the importance of physiologically balanced solutions for plants. I. Marine plants. Bot. Gaz. 42:127-134. 1906.

(100) _____. On the importance of physiologically balanced solutions for plants. II. Fresh water and terrestrial plants. Bot. Gaz. 44:259-272. 1907.

(101) _____. The nature of balanced solutions. Bot. Gaz. 47:148-149. 1909.

(102) Pember, F. R. Studies by means of both pot and solution culture of the phosphorus and potassium requirements of the barley plant. R. I. Agr. Exp. Sta. Bul. 169. 1917.

(103) Pirschle, K. Nitrate und Ammonsälze als Stickstoffquellen für höhre Pflanzen bei konstanter Wasserstoff-ionen Konzentration. Ztschr. Wiss. Biol. Abt. E. Planta 14:563-676 1931.

(104) Popp, H. W. A physiological study of the effect of light of various wave lengths on the growth of plants. Amer. Jour. Bot. 13:706-736. 1926.

(105) _____. Effect of light intensity on growth of soybeans and its relation to the auto-catalyst theory of growth. Bot. Gaz. 82:306-319. 1926.

(106) Prianischnikow, D. N. Ammoniak, Nitrate, und Nitrite als Stickstoffquellen für höhre Pflanzen. Ergeb. Biol. 1:407-446. 1926.

(107) Reed, H. S., and Haas, A. R. C. Some relations between the growth and composition of young orange trees and the concentration of the nutrient solution employed. Jour. Agr. Res. 28:277-284. 1924.

(108) Retunno, N. A. Effect of size of seed on plant production with special reference to radish. Bot. Gaz. 78:397-413. 1924.

(109) Rudolfs, W. Influence of temperature and initial weight of seeds upon the growth-rate of Phaseolus vulgaris seedlings. Jour. Agr. Res. 26:537-541. 1923.

(110) Saeger, A. Growth of duckweeds in mineral nutrient solutions with and without organic extracts. Jour. Gen. Physiol. 7:517-526. 1925.

(111) Salter, R. M., And McIlvane, T. C. Effect of reaction of solution on germination of seeds and on growth of seedlings. Jour. Agr. Res. 19:73-96. 1920.

(112) Sayre, J. D. The development of chlorophyll in seedlings in different ranges of wave lengths of light. Plant Physiol. 3:71-77. 1928.

(113) Schreiner, O., and Skinner, J. J. Nitrogenous soil constituents and their bearing on soil fertility. U. S. Dept. Agr. Bur. Soils Bul. 87:1-84. 1912.

(114) Sewell, M. C. Relation of the molecular proportions in the nutrient solution to the growth of wheat. Jour. Agr. Res. 28:387-393. 1924.

(115) Shirley, H. L. The influence of light intensity and quality upon the growth of plants. Amer. Jour. Bot. 15:321-322. 1928.

(116) Shive, J. W. A study of physiological balance of nutrient media. Physiol. Res. 1:327-399. 1915.

(117) _____. A study of physiological balance for buckwheat grown in three-salt solutions. N. J. Agr. Exp. Sta. Bul. 319:1-63. 1917.

(118) Sideris, C. P., and Krauss, B. H. The effect of sulfur and phosphorus on the availability of iron to pineapple and maize plants. Soil Sci. 37:85-97. 1934.

(119) _____, and _____, and Masunga, E. The effect of the salt concentration of the culture solution on the growth and composition of pineapple plants. Amer. Jour. Bot. 15:353-372. 1928.

(120) Spencer, E. L., and Shive, J. W. The growth of Rhododendron ponticum in sand sultures. Bul. Torrey Bot. Club 60:423-439. 1933.

(121) Stiles, W. On the relation between the concentration of nutrient solution and the rate of growth of plants in water culture. Ann. Bot. 29:89-96. 1915.

(122) _____. On the interpretation of the results of water culture experiments. Ann. Bot. 30:427-436. 1916.

(123) _____, and Jörgenson, I. Observations on the influence of aeration of nutrient solutions in water culture experiments, with some remarks on the water culture method. New Phytol. 16:181-197. 1917.

(124) Tarr, L. W., and Noble, S. C. The effect of hydrogen ion concentration upon the growth of seedlings. Del. Agr. Exp. Sta. Bul. 133:1-52. 1922.

(125) Theron, J. J. Influence of reaction on interrelations between the plant and its culture medium. Univ. Calif. Pub. Agr. Sci. 4:413-444. 1924.

(126) Tiedjens, V. A., and Blake, M. A. Factors affecting the use of nitrate and ammonium nitrogen by apple trees. N. J. Agr. Exp. Sta. Bul. 547:1-32. 1932.

(127) _____, and Robbins, W. R. The use of ammonia and nitrate nitrogen by certain crop plants. N. J. Agr. Exp. Sta. Bul. 526:1-46. 1931.

(128) Toole, E. H., and Tottingham, W. E. Influence of certain added solids upon the composition and efficiency of Knop's nutrient solution. Amer. Jour. Bot. 5:452-461. 1918.

(129) Tottingham, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. 1:133-245. 1914.

(130) _____, and Beck, A. J. Antagonism between manganese and iron in the growth of wheat. Plant World 19:359-370. 1916.

(131) _____, and Rankin, E. J. Nutrient solutions for wheat. Amer. Jour. Bot. 9:270-276. 1922.

(132) _____, and _____. The availability of iron in nutrient solutions for wheat. Amer. Jour. Bot. 10:203-210. 1923.

(133) Trelease, S. F., and Livingston, B. E. The relation of climatic conditions to the salt proportion requirement of plants in solution cultures. Science, n.s. 59:168-172. 1924.

(134) _____, and Trelease, Helen M. Relation of temperature to the physiological values of salt solutions as indicated by growth of wheat roots. Bul. Torrey Bot. Club 53:605-609. 1926.

(135) Waltman, C. S. Effect of hydrogen-ion concentration on the growth of strawberries in sand and in soil. Ky. Agr. Exp. Sta. Bul. 321:331-352. 1931.

(136) Willis, L. G., and Carrero, J. O. Rice investigations. Porto Rico Agr. Exp. Sta. Ann. Rpt. 1921:7-9. 1921.

(137) Wolfe, H. S. The Auximone question. Bot. Gaz. 81:228-231. 1926.

(138) Wolff, J. De l'influence du fer dans le développement de l'orge et sur la spécificité de son action. Compt. Rend. Acad. Sci. Paris 19:1022-1024. 1913.

(139) Yoe, J. H. 7-iodo-8-hydroxyquinoline-5-sulfonic acid as a reagent for the colorimetric determination of ferric iron. J. Am. Chem. Soc. 54:4139-4143. 1932.

(140) Zinzadzé, C. Nutrition artificielle des plantes cultiveés. III. Conditions d'emploi en milieu aqueux et mode de préparation des nouveaux mélanges nutritifs à pH stable. Ann. Agr. 3:53-72. 1933.